# GENETICS

*A periodical record of investigations bearing on heredity and variation*

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with the cooperation of

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VOLUME 44 JULY, 1959 NO. 4, PART 1

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VOLUME 44 JULY, 1959 NO. 4, PART 1

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## Effective with Volume 45, 1960

1. GENETICS will be changed from a bimonthly to a monthly journal.

2. The annual volume of the journal will include a minimum of 1,000 pages. The Editors expect to include enough additional pages to print all acceptable manuscripts if funds are available.

3. The subscription rate will be $12.00 for a complete volume (January-December). Foreign postage will be $1.00 extra. Single copies will cost $1.50.

4. Contributions to GENETICS may be in the field of genetics proper, or in any related scientific field if the work reported is primarily of interest to geneticists.

An author is expected to submit to the Editors two typewritten copies of a manuscript, including an original typed copy. Manuscripts must be double spaced throughout, including the footnotes, tabular material, legends and Literature Cited.

5. Each paper published will show the date that the manuscript was received at the Editorial Office. Ordinarily, papers will appear in print in the order they were received as manuscripts, but manuscripts will be classified according to whether they are short or long and the two will be processed on different printing schedules.

a) Short papers are those which are no more than four printed pages in length, including tables and figures. They must conform to the standards and to the general usage in GENETICS, and will be reviewed for quality and scientific merit. Every effort will be made to publish short papers at an accelerated rate. Hence they may appear out of order of date of receipt in comparison to long papers. If a paper is more than four printed pages in length, it will be treated as a long paper in the printing schedule.

b) Long papers are those which are longer than four and up to a maximum of 20 printed pages including tables and figures. They will be accepted for printing as in the past. Each monthly issue of the journal will include both long and short papers.

Papers longer than 20 printed pages will not be accepted for printing except by special vote of the Editorial Board. The author will be expected to pay the costs for printing and handling the extra pages. The additional pages will increase the length of the volume rather than replace another paper. An author who submits a long paper can expect a delay in printing of the paper because of the extra time needed for processing by the reviewers, the Board, and the Editors.

6. In order to help us expedite the printing schedule, an author should prepare his manuscript according to the following instructions. Failure to do so can lead to delay of appearance of the paper in print and may result in charges against the author for extra printing costs.

a) Manuscripts must conform to the general usage in GENETICS, particularly in regard to references to literature, arrangement of Literature Cited, and inclusion of a Summary.

b) A gene symbol should be defined or identified with the mutant character the first time the symbol is used in a manuscript.

c) Excessive footnotes are to be avoided. Footnotes to text statements may be included in the text (parenthetically if necessary).

d) Tables must be typewritten on separate pages with double spacing throughout, and must be arranged to conform to journal page size (5 x 7⅜).

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# ABSTRACTS OF PAPERS PRESENTED AT THE 1959 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

## University Park, Pennsylvania, August 31 to September 2, 1959

Abrahamson, S., and L. P. Fanale, Rutgers University, Newark, N.J.: *The Induction by anoxia of melanotic masses in Drosophila.*—Prolonged anoxia (1–2 hours) was discovered to produce large melanotic masses, which resemble the tumors described for Drosophila by other investigators. These masses develop in the anterior abdominal region from within minutes after the treatment to about 48 hours later. No flies of the genotypes studied were ever observed to have melanotic masses except when produced by treatment. The flies bearing the masses become increasingly feeble and with rare exception die within two days.—Preliminary experiments have shown that several variables significantly influence the frequency of melanotic response. These are: (1) genotype (a range of 2 to 80% was obtained with six genotypes examined); (2) sex (females are more susceptible than males); and (3) age (0–12 hour adults demonstrate the lowest frequency)—Studies are in progress to investigate the nature and development of the induced melanosis. (This work was supported by a grant from the National Science Foundation and the Rutgers University Research Council.)

Adelberg, Edward A., and Sarah N. Burns, (Introduced by I. Michael Lerner), University of California, Berkeley, Calif.: *A variant sex factor in Escherichia coli.*—The sex factor (F) of *Escherichia coli* belongs to the class of genetic determinants called episomes: determinants which can exist alternatively as cytoplasmic particles or as attached chromosomal elements. The origin and properties of a variant F will be described.—The wild type sex factor ($F^1$) of strain K-12 is characterized by its low affinity for the chromosome and its lack of any preferential site of attachment. The variant sex factor, $F^2$, arose in Hfr strain P4x, in which $F^1$ had been stably attached to the chromosome between the loci *prol* (controlling proline synthesis) and *lac* (controlling lactose fermentation). In oriented chromosome transfer by P4x, *prol* is the first marker to be transferred and *lac* is the last; the attached sex factor is linked closely to *lac*.—Strain P4x gave rise spontaneously to strain P4x-1, in which the sex factor has acquired two new properties: its attachment to the chromosome is unstable, kinetic studies of zygote formation indicating a rapid alternation between the attached and cytoplasmic states; and it exhibits a high and specific affinity for the site at which $F^1$ had been fixed. The latter property is recognized by the fact that infection with $F^2$ converts $F^-$ cells into donors which transfer their chromosome at high frequency and with the same orientation as Hfr strain P4x. It is proposed that $F^2$ arose by genetic exchange between attached $F^1$ and chromosome; the incorporation of chromosomal material would explain the high affinity of $F^2$ for the original site of attachment.

Alexander, Mary L., Biology Department, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas: *Mutation rates at specific gene loci with different total doses of X-radiation.*—The mutation rates at the autosomal loci of *ru h th st* $p^p$ *cu sr* $e^s$ on the third chromosome of *Drosophila melanogaster* have been tested for mature sperm using 900r of X-radiation. Twenty-three mutations were recovered in a sample size of 54,683. The average rate of $5 \times 10^{-5}$ per locus with 900r is slightly less than one third the rate of $17 \times 10^{-5}$ per locus with 3000r. Mutation rates for visible mutations at specific gene loci increased in a linear manner with an increase in dosage. The average rates per r per locus were $5.7 \times 10^{-8}$ with 3000r and $5.8 \times 10^{-8}$ for 900r. With 900r, 40% of the mutations were lethal or semi-lethal; with 3000r, 60% were lethal or semi-lethal. Fifty percent of the mutations were associated with chromosome aberrations with 3000r but only 20% of the mutations showed aberrations with 900r.—Com-

parisons of mutation rates in sperm and spermatogonial cells with 900r are more exact than in previous tests. The average mutation rate for spermatogonial cells of $1.5 \times 10^{-8}$ per r per locus has been calculated using 15 independent, cluster mutations. The 95% confidence limits do not overlap those for the rate with mature sperm. A comparison of mutation rates based on point mutations (mutations not associated with chromosome aberrations) shows little or no overlap in the confidence limits of the mutation rates in sperm and spermatogonial cells. Selection of chromosome aberrations from spermatogonial cells is indicated by their absence in the gonial tests. The higher mutation rate for sperm using point mutations indicates, in addition, sensitivity differences in the two types of cells. (This work was supported in part by Contract AT(40–1)–1828 with the U. S. Atomic Energy Commission.)

ALTENBURG, EDGAR, and L. S. ALTENBURG, Rice Institute, Houston, Texas: *Comparative crossover rates in the trans and cis arrangements of alleles at the white locus in Drosophila.*—Studies are being made of the relative crossover rates in flies heterozygous for apricot and white in the trans and cis arrangements, using LEWIS' attached-X stock with *Cy* and *Ubx* inversions in the second and third chromosomes to enhance the crossover rates at the white locus; and also the rates in flies heterozygous for Brownex and apricot in JUDD's unattached-X stocks. Results to date indicate that there may be a significantly higher crossover rate ($P = .05$) from trans to cis arrangement than from cis to trans arrangement in the LEWIS stock, based upon the finding of 28 females with the cis arrangement among 170,000 females with the trans arrangement (a rate of $0.0165 \pm 0.0031\%$) as compared with five cis females among 58,000 trans females ($0.0074 \pm 0.0033\%$). In the JUDD stock, however, the rate from trans to cis, although larger, is not significantly greater than that from cis to trans, based on 39 cis females found among 426,000 trans females (a rate of $0.0092 \pm 0.0015\%$) compared with two trans females among 43,000 cis females (a rate of $0.0046 \pm 0.0034\%$). These rates, if confirmed by more extensive data, would indicate structural heterozygosity at the white locus. In the case of the Brownex-apricot heterozygotes, the white "exceptions," previously reported by JUDD, were recovered, which would also indicate structural heterozygosity at this locus. (This work has been supported by a grant from the National Institutes of Health, U. S. Public Health Service.)

ALTENBURG, L. S., and EDGAR ALTENBURG, Rice Institute, Houston, Texas: *The mutagenicity of 2,5-bis-ethyleneimino hydroquinone in Drosophila.*—About .001 ml of an aqueous suspension of 2,5-bis-ethyleneimino hydroquinone in concentrations varying from $5 \times 10^{-3}$ M to $5 \times 10^{-2}$ M was injected into the abdomens of young males of MULLER's Maxy stock, the females of which are heterozygous for 13 recessive visible mutations in the X chromosome. Over 12,000 daughters of 125 injected males were examined for "Maxy" visibles, and in addition more than 1,000 of the daughters were tested individually for the presence of lethals. A total of 16 visible Maxy mutants were recovered. Of these, 11 had the distinctive light apricot eye color possessed by Maxy eye-color mutants when in a Maxy female homozygous for vermilion. There were also two *f*, one *y* mosaic, one *odsy*, and one *ct*. Of the 16 visibles found, only three proved to be transmissible and viable (*g*, *f*, *y*), four were associated with lethals, and nine were sterile, died before they could be tested, or failed to transmit the mutant trait. The number of transmissible and viable Maxy visible mutations plus those associated with lethals in the treated series is therefore 7 in 12,000, or a rate of $0.058 \pm 0.022\%$, compared with 8 in 151,000, or a rate of $0.005 \pm 0.002\%$, found in the control series, the difference in rates being statistically significant. A lethal rate of $3.0 \pm 0.5\%$ from the control males, as compared with a control rate of $0.2 \pm 0.1\%$, was obtained. For the treated series, this indicates a ratio of one visible mutation to 12 lethals.—2,5-bis-ethyleneimino hydroquinone acts partly as an alkylating agent, and as a hydroquinone it participates in oxidation-reduction reactions. It might also generate free radicals. These data indicate that this compound is more mutagenic in our hands than some compounds that act simply as alkylating agents, in particular phenylalanine mustard and myleran, which themselves are rather highly mutagenic. (This work has been supported by a grant from the National Institutes of Health, U. S. Public Health Service.)

ARONSON, MARGARET M., (Introduced by R. C. LEWONTIN), Department of Biology, University of Rochester, Rochester, N. Y.: *New evidence for genetic activity of the Y chromosome in D. melanogaster.*—Segregation of the separated arms of III in the heterozygote between T2(1:3)$^{K}$ (*Drosophila Inform. Serv.* 30:76, 1956) and T3L.4L;4R.3R (Dubinin 2) has been studied. The point of breakage is in the chromocentral region of 3R in the T(1:3) stock; of 3L in the other. Four expected classes are recovered in statistically equal numbers, thereby adding these data to the accumulating evidence that the centromere itself does not play an active role in segregation. One class is deficient for the region of the III centromere, and this deficiency is lethal when homozygous. Both males and females heterozygous for this deficiency are fully viable and normal. Deficient males which are X0 and haplo-IV are not viable, but in the presence of the Y chromosome deficient haplo-IV males are viable. The viability of this last class indicates that the Y chromosome is a genetically active chromosome and can compensate in some way for an autosomal deficiency.

ATZ, JAMES W., New York Aquarium, New York Zoological Society, Brooklyn, N.Y.: *Genetically controlled atypical pigment cell growth among the hybrids of six species of xiphophorin fishes.*—Four of the six currently recognized and described species of xiphophorin fishes are polymorphic for pigmentary patterns formed by macromelanophores. These cells are characterized by their size, their *spotted* phenotypes and their tendency to multiply excessively, producing melanoses and melanomas, when introduced into foreign genotypes through hybridization. All nine known macromelanophore patterns are inherited as autosomal or sex-linked dominants. Six of the genes, in *Xiphophorus maculatus* and *X. variatus*, appear to be alleles; the rest occur on non-homologous chromosomes. Hybrids involving 26 of the 45 possible combinations have been produced. Interactions range from extreme melanosis with melanomas present in many of the melanotic fish to complete suppression of pattern. The genes for macromelanophore patterns may be ranked according to their potency in producing excessive pigmentation in the various hybrids, while the species may be ranged according to their "resistance" or "susceptibility" to atypical pigment cell growth. Some genes have shown no capacity for atypical growth; others do so in every hybrid combination. Especially noteworthy is the observation that the species (*X. maculatus*) which has the most potent macromelanophore genes is the only one whose hybrids have never shown enhanced *spotted* patterns. The situation is best understood by reference to MYRON GORDON's (1950, 1958) hypothesis of how genes for macromelanophore patterns became integrated into the genotype of the species. This, in turn, indicates some similarity in the biochemical processes of the nine genes. Nevertheless, one *spotted* pattern may be enhanced while another is simultaneously suppressed in a single hybrid.

BAND, HENRETTA T.,[1] D. E. SHEPPARD, and P. T. IVES, Amherst College, Amherst, Mass.: *A survey of second and third chromosomes from the South Amherst population of Drosophila melanogaster.*—273 second and 252 third chromosome collected in September 1958 were analyzed by *Cy* and *TM1*, respectively. The second chromosomes were 35.9% lethal or semilethal (less than 17% +); 43.2% quasinormal (18 to 29% +); 17.4% normal (30 to 33% +) and 3.5% supernormal (34 to 42% +). The comparable third chromosome frequencies were, respectively, 40.1%, 38.5%, 14.3% and 7.1%. Differences between these two distributions as determined by chi-square are not significant. Significantly more third chromosomes than seconds carried visible recessive mutations with *e* and *bw* appearing most frequently.—After a period of cool weather 151 second and 124 third chromosomes were collected in October and showed a significant decline in lethals and semilethals to 25.8% in both second and third chromosomes. Nearly complete tests show a low frequency (under 0.5%) of allelism among the second chromosome lethals in each collection, indicating that neither was composed of flies migrating from one small breeding unit. Third chromosome lethal allelism tests are underway.—The decreased October lethal frequency has not previously been observed here. This observation suggests that the decline in frequency between the peak of one season and the beginning of the next in the following summer takes place late in the breeding season. The low percentage of allelism among the lethals in the

two collections suggests that the October decline may be due to dominant selection against lethal chromosomes rather than to loss from homozygosis. ([1] Public Health Service Research Fellow of the National Cancer Institute.)

BEATTY, A. V., and JEANNE W. BEATTY, Emory University, Atlanta, Ga.: *Postirradiation modification of chromosomal aberration yield.*—In Tradescantia microspores, the yield of X-ray-induced chromosomal aberrations can be modified by postirradiation treatment. At 30° C and at 50r/min, material given 200r in and posttreated in air for 15 min yielded 0.23 aberrations per cell; posttreated in helium for 15 min gave 0.30 while posttreatment for 60 min in helium gave 0.40. A total dose of 400r given material in and posttreated for 15 min in air yielded 0.79 aberrations per cell, while 60 min posttreatment in helium gave 0.98. At 40° C and at 50r/min, material given 200r in and posttreated in He for 15 min yielded 0.20 aberrations per cell, while posttreatment in air for 15 min gave only 0.11. A total dose of 400r with posttreatment in helium for 15 min gave 0.66 aberrations, while posttreatment in air gave only 0.26. These post-X-irradiative effects can be explained and predicted on an energy-supplying basis. Similar experimental procedures, combined, however, with fractionated doses, gave results which indicate that the rejoining time for X-radiation-induced chromosome breaks is the same for material irradiated in either helium or an oxygenated atmosphere.

BECKER, H. J., (Introduced by CURT STERN), University of California, Berkeley, Calif.: *Variegation in the zeste alleles of Drosophila melanogaster.*—Of the alleles $z$, $z^a$, $z^m$ and $z^l$ the two latter ones show a variegation pattern characteristic for cell lineage in both males and females, also in compounds with the two former alleles. In flies raised in favorable temperatures, which are different for each allele and each sex, the spots are normal red or slightly darker than normal; they contain the red and the brown pigment component. The background of the eye is lemon; here the red component is missing. The size and distribution of the red spots were compared with X-ray induced mosaic spots, whose size depends on the age at the time of irradiation. It can be concluded that the time of determination as to whether the descendants of a cell develop red pigment or not, takes place at the end of the first larval instar. Of the roughly 20 cells, of which the presumptive eye area consists at this time of development, any number can be determined to red pigment formation in any individual head anlage, on the average 20–25% in $z^m$ males, probably slightly less in $z^l$ males, 5% in $z^m/z^l$, 2–3% in $z^m/z^m$ and $z^l/z^l$, less than 2% in $z^m/z^a$, $z^m/z$, $z^a/z^l$ and $z/z^l$ and none in $z/z$ and $z^a/z$ females. This newly acquired property is handed down to the daughter cells. The site of the red pigment formation, the primary pigment cells, does not appear until about nine cell divisions after the determination. It seems likely that the determination is imposed upon the nucleus itself. (This study was aided by a grant from the National Science Foundation.)

BIANCHI, A., Institute of Genetics, University of Milan, Milan, Italy: *A new type of chromosome 7 in Italian maize.*—A quasiuniversal characteristic of chromosome 7 makes its identification easy in most American maize. On the long arm, adjacent to the centromere, the chromosome is broad and stains deeply, following aceto-carmine technique on pollen mother cells. Such a deep staining region seems, on the contrary, undetectable in most Italian maize. (So far more than two hundred samples from all over Italy have been carefully studied, and about ¾ of the plants show no deeply staining region). This may be an aspect of the scarce endowment in heteropycnotic material present in Italian maize. Moreover, on the long arm, the typical large knob is often replaced by a small one followed closely by a very prominent chromomere. Such structure is estimated present in about ten percent of the examined plants. It is undecided whether such two prominent bodies result from a splitting of the heterochromatin of the common knob or rather whether a small knob follows the regular one of reduced size.

BLUMEL, JOHANNA, Department of Surgery, Orthopedic Division, University of Texas, Medical Branch, Galveston, Texas: *A combination of congenital cataracts and cerebral palsy in a brother and a sister.*—While investigating the hereditary aspects of cerebral palsy at the Moody State School for Cerebral Palsied Children, a family group with two affected children, a boy and a girl, came to our attention. In addition to spasticity, these children were also afflicted with congenital cataracts. A genetic and metabolic study of the family was undertaken. Results of this investigation, including electrophoretic and chromatographic studies on the body fluids of these individuals will be presented and discussed.

BOSE, SMRITIMOY, Blandy Experimental Farm, University of Virginia, Boyce, Virginia: *Fusion and fragmentation of chromosomes and the mechanism of karyotype evolution in Lycoris.*—Somatic numbers reported for Lycoris are, 12–17, 22, 27, 29 + 1B, 30 and 33. Karyotype analyses show V and rod-shaped chromosomes. While no taxa have all V's, some have all rods and others have a mixture of V's and rods. Taxa having only rods have chromosomes with subterminal constrictions only. Among those forms with both V and rod chromosomes, some have rods of the nearly terminal constricted type only, others have both subterminal and nearly terminal constricted rods; still others have rods of only subterminal constricted type. Intraspecific chromosomal polymorphism is shown by those forms which have rods of only nearly terminal constrictions. Intra and interspecific chromosomal polymorphism without the addition of extra chromosomal elements seems to be characteristic of karyotype evolution in Lycoris.—Present knowledge of centromeric structure and of telocentric chromosomes have been utilized in seeking an explanation of the mode of origin of chromosomes. Hybridization, translocations, and polyploidy are among the other factors which also obviously have played a part. Either fusion of two rods to form a V; or the fragmentation of a V to form two rods, could be taken as the essential mechanism for karyotype evolution and speciation in Lycoris. Depending on the acceptance of one over the other, a phylogenetic decrease or increase in basic number could be visualized involving either a basic set of six V chromosomes or 11 all of which were rods.

BUCHERT, J. G., Yale University, and the Connecticut Agricultural Experiment Station, New Haven, Conn.: *The effect of critical time and location of gene action on the degree of pollen abortion and the inheritance pattern in the restoration of cytoplasmic male sterility in maize.*—As a rule, progenies reflect Mendelian inheritance of the genes *(Rf)* capable of suppressing the cytoplasmic male-sterile factor ([*ms*]). Cases where this was not so were investigated. All crosses (or selfs) of the type $[ms_2] \times [ms_2]Rf^2rf^2$ yielded only fertile progeny; while $[ms_2]Rf^2rf^2 \times rf^2rf^2$ gave segregation. Thus, when with $[ms_2]$, $Rf^2$ was selected for in the pollen. When the inheritance of $Rf^2$ was checked in plants not having $[ms_2]$, no such selection occurred. Therefore, the selection for $Rf^2$ and consequently the inheritance pattern was dependent upon an interaction between $[ms_2]$ and $Rf^2$ in the presence of the residual genotype used (A158, M14, and P39).—The degree of pollen abortion has also been determined by the interaction. In contrast to cases where progeny segregation is normal, microscopic observation of $[ms_2]Rf^2Rf^2$ and $[ms_2]Rf^2rf^2$ plants revealed that while homozygotes had as high a percentage of normal pollen as the corresponding inbred, the heterozygotes made pollen 50 percent of which aborted.—It has been concluded that: (1) the critical time and place of production of the primary diffusible gene product was in the gametophyte, shortly after the formation of crosswalls during microsporogenesis; and that the restoration process took place shortly thereafter at the same place; (2) the critical stage determining the pollen abortion occurs after microsporogenesis, shortly before the deterioration can be seen; (3) a small particle-number hypothesis cannot account for the results; (4) the tapetum is not involved in the abortion process to the extent of withholding food, resulting in starvation of the spores.

BURDICK, A. B., T. MUKAI, and M. E. KRAWINKEL, Purdue University, Lafayette, Ind.: *The genetic effect of low doses of irradiation in heterozygous and homozygous Drosophila lines; the*

*hypothesis of wild type iso-allele mutation.*—When low-dose (25–150r) X-irradiated genomes from a homozygous line are tested for heterozygous viability in the homozygous background in which they were produced, they give evidence of having suffered many more dominant viability affecting mutations than can be accounted for by the "standard" $5 \times 10^{-8}$/r/gene mutation rate. The mean heterozygous viability of such genomes is usually higher than control and the variance between genomes is significantly higher, indicating that the induced mutations in heterozygous condition are somewhat more likely to improve than to diminish viability of a homozygous genotype. Random, plus and minus, wild type isoallele mutations in high frequency (ca. $1 \times 10^{-6}$/r/gene) can account for these results on a basis compatible with mutation and evalutionary theory.—If the iso-allele hypothesis is true, we would expect that such mutations in heterozygous condition, tested in a variable *heterozygous* background, would have no effect on the mean or variance of viability. We irradiated males from a CMI-produced homozygous line, balanced and duplicated the irradiated autosomes in males with T(2–3) Xa (isogenic with the irradiated homozygous line) and tested them, replicated in duplicate, by crossing to the homozygous line. Mean and variance increases were highly significant. Exactly the same experiment was conducted starting with irradiated males from a random mating population, unrelated to the homozygous line against which the irradiated autosomes were tested. Means were the same as control; some variances were slightly less than control.

Capenos, Mary Rowan, and E. B. Spiess, Department of Biological Sciences, University of Pittsburgh, Pittsburgh 13, Pennsylvania: *Origin of lethality in certain second chromosome recombinants of Drosophila persimilis.*—Crosses involving particular second chromosomes which had produced lethality, poor average viability, or high variance for viability by recombination were repeated (42 out of the 190 crosses reported in GENETICS 44 by Spiess). Recombinant chromosomes were made homozygous by the marker technique. Out of 16 original lethal-producing crosses only one gave lethals in the repeat; out of 14 original low viability crosses four produced lethals in the repeat; and out of 12 high variance crosses two gave lethals. Mean viabilities and variances of the nonlethal chromosomes of repeat crosses did not differ significantly from those of original crosses, indicating a constancy in recombinational effects. Flies balanced with a lethal chromosome obtained from recombination were crossed out to a strain with a good viability chromosome. If the lethality is due to interaction of linked loci which are far enough apart to have better viability restored by crossing over, then recombinant chromosomes derived from $F_1$ females should depart from the 50:50 segregation ratio by giving an excess of viable chromosomes. Non-recombinant chromosomes derived from $F_1$ males, in which crossing over is suppressed, were tested as controls. In these "desynthesis" crosses, the number of nonlethal recombinant chromosomes from $F_1$ females was significantly increased in five out of seven lethal crosses tested. However the same excess occurred in chromosomes tested from $F_1$ males. Departure from expected segregation values could result from low penetrance of a lethal mutation, selection for non-lethal bearing gametes, or a mechanism affecting segregation itself to produce an excess of nonlethal gametes. Thus desynthesis, a criterion for distinguishing recombinant from mutational lethals, cannot be proved from these data. Two of the seven lethal producing crosses were later shown to contain mutational lethals. The fact that specific chromosomes can recombine to give repeatable viability modifications indicates that genic interaction on the second chromosome of *D. persimilis* exists but apparently the interaction is not of sufficient magnitude to produce lethality by recombination. (Work supported under Contract AT(30–1)–1775, U. S. Atomic Energy Commission).

Carlson, E. A., and J. L. Southin, Queen's University, Kingston, Ontario, Canada: *Preliminary genetic evidence supporting the complementary structure of the hereditary material in Drosophila spermatazoa.*—Females carrying the markers echinoid, dumpy, and clot (*ed dp cl*) were inseminated with mature *Ore R* wild type sperm and exposed to a single X-ray dose of 4000 roentgens. Among the numerous induced mutations found at the dumpy locus was a single female on whose right side appeared an oblique wing phenotype, but which was other-

wise normal, and on whose left side appeared a more extreme oblique wing, thoracic vortices, and reduced leg size. The female, bred to *ed dp cl* males gave two mutant progeny—an oblique type mutant ($dp^{o}$) and and a truncate type ($dp^{olv}$). These were designated $o^{bm}$ and $olv^{bm}$, respectively, after further testing for the presence of the lethal expression with other members of this series. Preliminary pseudoallelic analysis indicates $o^{bm}$ and $olv^{bm}$ both capable of recombination with the right-most member of the dumpy series ($v^{2}$). Of particular significance is the proximity of these two mutants in the order established or suggested for this series: *l ol olv o lv ov v*, where the four right-most members were directly determined by recombination in the appropriate intervals and the four left-most members were assigned their map positions with respect to one another by their differences in recombination rates when each was tested in heterozygous compound with *ov*. Both the double mutant and the wild type recombinants were obtained from the $o^{bm}/ed\ v^{2}\ cl$ series, and wild type recombinants were obtained from the $olv^{bm}/ed\ v^{2}\ cl$ series. Implications of this and other mosaic mutations for the interpretation of the genetic material in the mature spermatozoan and its relation to the mutation process will be discussed. (This work was supported first by Contract AT(11–1)–195, granted by the AEC for work of H. J. Muller and associates at I.U., and then by grant G-7621 allotted by the National Science Foundation (USA) to the senior author at Queen's University.)

Carson, H. L., Washington University, St. Louis, Mo.: *Effect of irradiation on artificial populations under strong natural selection.*—Four replicate populations of *se ss* $e^{s}$ *k ro* stock of *Drosophila melanogaster* were run in vial populations in which food, space and change schedule were rigidly controlled, producing stringent natural selection. The usual biomass of about 90 mgs wet weight was maintained under the control conditions. Irradiation was begun on two of these populations in June 1958 and is continuing at intervals, to date. Imagoes of all ages received 1000r of X-rays/wk in a single dose for 20 successive weeks. Radiation was then suspended for ten weeks, following which additional 10-week periods of radiation were alternated with 10-week periods of relaxation of radiation. Each experimental population has thus far received 30,000r. The irradiated populations declined precipitously in numbers but less in biomass. Thus the mean size of the flies in the irradiated populations increased sharply; this appears to be a simple effect of decreased competition for food. During the two periods of relaxation of radiation, the experimental populations have quickly risen again to the level of the controls but have not surpassed them.

Chu, Ernest H. Y., and Norman H. Giles, Yale University, New Haven, Conn.: *Qualitative and quantitative analyses of X-ray-induced human chromosome aberrations in cultures of normal diploid somatic cells.*—The present report is concerned with studies of the types and frequencies of X-ray-induced aberrations in human chromosomes. Normal diploid human cells actively proliferating at 37° C *in vitro* were irradiated with X-ray doses between 25 and 100r, at constant intensity. Cytological examinations of chromosomes were made at successively later intervals after irradiation, utilizing well-spread, colchicine-blocked metaphases. Analyses have been made of over 1300 intact, euploid, experimental and control cells. Previous studies had led to the construction of a detailed idiogram of the human karyotype, permitting the recognition of individual chromosomes—a circumstance often essential for the proper interpretation of aberrations. Qualitative analyses indicate that two major categories of aberrations—chromatid and chromosome—are present in irradiated cells. The majority of the various types of chromatid and chromosome aberrations characteristic of classical materials, such as Tradescantia microspores, have been detected. Chromatid types constitute the only aberrations in cells observed shortly after irradiation (e.g., 18 hours). At a later period (e.g., 25 and 30 hours), chromosome aberrations appear and a mixture of types is present. Finally (e.g., 50 hours), only chromosome aberrations are found. A general decrease in radiosensitivity (as judged by aberration frequencies) occurs in conjunction with the temporal shift from cells containing chromatid aberrations to those containing chromosome aberrations. Present quantitative data for both the chromatid and chromosome aberration types indicate the existence of two major kinds of dose-effect relation-

ships. In general, terminal deletions increase linearly with dose, whereas exchanges exhibit an exponential relationship compatible with a two-hit curve.

COE, E. H., U. S. Department of Agriculture and University of Missouri, Columbia, Missouri: *Time of the regular conversion-type event at the B locus in maize.*—Experiments reported previously have shown that *B′* regularly causes *B* to change to *B′* but that *b* is unaffected by B′. A mutable allele at the *B* locus, $B^v$, simulates *b*, but mutates frequently to *B* expression. When $B^v$ is confronted with *B′* the *B*-like capacity of $B^v$ is unaffected: heterozygous plants have intense sectors.—Either $B^v$ and its *B* mutants are intractable, or conversion occurs at or near meiosis and *B′* suppresses *B* action throughout the life cycle (but only when the two are together from the time of fertilization). Results of irradiation experiments also have a bearing on the time of the conversion-type event.

CRAWFORD, I. P., and C. YANOFSKY, Stanford University, Stanford, California: *Protein alterations associated with suppressor mutations and reversions in Escherichia coli.*—In *E. coli*, tryptophan synthetase, the enzyme system catalyzing the final step in tryptophan biosynthesis, consists of two protein components termed A and B. The gene controlling protein A is designated *tryp-2;* it is closely linked to the gene *tryp-1* which controls protein B. Point mutations within these genes have been observed to cause abnormalities of two types, (1) lack of the component and any protein immunologically related to it, or (2) the production of a protein (called cross reacting material or CRM) immunologically similar to the normal enzyme but altered with respect to enzymatic capability. These two mutational types have been obtained in about equal numbers.—Starting with a strain having a CRM-producing mutation of the *tryp-2* gene, an investigation of the nature of the protein or proteins produced following a second mutation toward prototrophy was undertaken. In these experiments both true revertants and suppressed mutants were obtained. The most interesting finding resulted from examination of one suppressed mutant. In this strain it was possible to identify and partially separate two A proteins. One of these was identical with the altered component A of the original mutant strain. The other shares several properties, at least, with normal component A. Thus in this case suppression has resulted in the appearance of a new, enzymatically effective protein in addition to the altered one formed in the unsuppressed mutant. The results of similar experiments with revertant strains will be presented.

DATTA, S. P., W. H. STONE, W. J. TYLER and M. R. IRVIN, University of Wisconsin, Madison, Wis.: *A possible heritable exception in cattle blood groups.*—The B locus of cattle determines the expression of over 160 combinations (phenogroups) of about 21 antigenic factors of the erythrocytes. A bull of gentoype $GY_2E'_1/—$ (dash represents the absence of detectable factors) in a mating with a cow of genotype $QE'_1I'/D'E'_3$ produced a female calf whose blood showed a previously unrecognized $GE'_1$. The calf's probable genotype was $GE'_1/D'E'_3$. The calf inherited the phenogroup $D'E'_3$ from the dam and the exceptional phenogroup $GE'_1$ presumably from the sire. Illegitimacy was improbable because otherwise the blood groups of sire, dam, and calf were genetically compatible, and no other bull in the herd could qualify as the sire.—A variety of serological tests failed to detect $Y_2$ on the erythrocytes of this calf. Detection of $Y_2$ in the blood of the sire, a full sib and in other cattle heterozygous for phenogroups containing $Y_2$ excluded allelic interaction as the cause of this exceptional phenogroup. The failure to detect any serological differences in the $Y_2$ factor of the phenogroup $GY_2E'_1$ in other cattle of this line bred herd indicates that this exception is not due to modifiers. One plausible explanation would be a genetic change in the sperm of the sire. Similar exceptions in the B phenogroups have been interpreted as mutational by STORMONT (Am. Naturalist 89:105, 1955). Offspring of this exceptional individual have failed to establish the genetic or nongenetic nature of this exception. The first calf was slaughtered before it could be blood typed, while three other calves inherited the alternate phenogroup $D'E'_3$, from the dam.

De Giovanni, Rosalie, and Stephen Zamenhof, Department of Biochemistry, Columbia University, New York, N.Y.: *The effects of deuterium oxide on Escherichia coli.*—The inhibitory effects of deuterium oxide ($D_2O$) on various organisms have been frequently reported. Early experiments with Drosophila (Zamenhof and Demerec 1943, Am. Naturalist 77:380) did not demonstrate a significant increase in the mutation rate. Similarly, the replacement of exchangeable hydrogen atoms by deuterium upon dissolving the transforming principle in $D_2O$ did not change the transforming activity in *Hemophilus influenzae*. However, recent progress in the microbial genetics made it suggestive to conduct further study.—Distilled $D_2O$ (99 percent in nutrient broth) prolonged four times the division time and lowered four times the final cell count of *Escherichia coli* mutant strain I; parent strain 15 was totally inhibited. Each of the three other strains tested exhibited a different response.—The cultivation of strain I in 99 percent $D_2O$ nutrient media resulted in the isolation of a *spontaneous* rough mutant (I/D) which was more resistant to $D_2O$: colonies in $D_2O$ medium contained six times more cells than in strain I. The spontaneous mutation rate to $D_2O$ resistance was of the order of $10^{-4}$; it could be increased 300 times by ultraviolet irradiation.—In addition to I/D, *$D_2O$-induced* mutants were isolated. Some mutants (frequency one percent) were characterized by small size ("pinpoint") colonies; they were unstable and reverted to normal size colonies with a high mutation rate. Normal growth on richer media suggests the nutritional nature of such mutants. Another mutant (frequency 0.02 percent) was unstable auxotroph (back mutation rate $10^{-3}$). (Supported by a grant from the Atomic Energy Commission).

DeLamater, Edward D., University of Pennsylvania, Philadelphia, Pa.: *Observations on the organization of the isolated bacterial chromosome.*—Nuclei have been isolated from cells of *Bacillus megaterium*, following treatment with sodium glycocholate. Chemical analysis indicates that the isolated structures contain DNA, RNA and protein. Cytochemical evidence likewise indicates the presence of DNA, RNA and –SH–containing protein. It has been possible to identify these structures giving cytochemical reactions in the electron microscope (using the Philips EM–75), and to define what appears to be the chromosome number, and the paired state of the chromatids in prophase and metaphase. Exposure of isolated chromosomes to phosphate buffer, following graded periods of sonication, causes progressive disruption of the integrity of the nuclear and chromosomal structure, causing the chromosomes to appear ragged or tattered. Using the RCA EMU III-D electron microscope at low power, a comparable picture to that observed with the Philips EM–75 is obtained; with higher magnification, after short exposure to sonication, the individual chromosomes appear to be composite multistranded structures in which longitudinal beading can be observed. With increasing periods of sonication, the structures are progressively disrupted, further suggesting that the chromosomes are made up of multiple longitudinal strands of perhaps relatively short length held in pole-to-pole arrangement. In material that has been more completely disrupted, a longitudinal stranded structure is visible, with associated delicate cross fibrils and granules which approximate the size of RNA molecules.

These constitute preliminary observations, and no effort toward an ultimate interpretation has yet been made. Photographic evidence will be presented.

De Marinis, F., Fenn College, Cleveland, Ohio: *Frequency of somatic crossing over in various parts of the body of Minute-n Drosophila.*—Female flies *M(1)n/yw sn*$^3$ were observed for mosaic areas for yellow-singed setae on the body and head, and for white facets in the eye. These areas are indicative of somatic crossing over. A detailed study on the frequency of mosaics in various regions of the body was made; head, eye, thorax, each pair of legs, each tergite, and each sternite. A total of 322 mosaics was found in a total of 2644 females inspected. The frequency of mosaics ranged between .05%, in the sixth sternite, and 2.80%, in the thorax region. This indicates a considerable range of frequency for this genetic event for various parts of the body. However, the various parts of the body do not have equal surface areas, nor do they have an equal number of bristles and hairs (setae). Therefore, surface area measurements and seta-counts were made in each part of the body. These two factors are considered in determining the

true frequency of these mosaics. In brief, the actual number of mosaics found are: head—71 (2.69%), thorax—74 (2.80%), and abdomen—145 (5.48%). The calculated number of mosaics (taking into account the size of the area and the number of setae in each area) are: head—136 (5.15%), thorax—151 (5.70%), and abdomen—145 (5.48%). These estimates indicate that the frequency of somatic crossing over is approximately uniform in various parts of the body of these Minute-n flies.

Doane, W. W., Yale University, New Haven, Conn.: *Developmental physiology of a female sterility mutant in D. melanogaster.*—The developmental and physiological effects of a recessive factor, female sterile-adipose (*fs-adp*), located at 2–83±, are being analyzed. An attempt is being made to relate the lethality found in eggs laid by homozygous females with an abnormal fat metabolism, characteristic of the adults. Evidence indicates that, under certain conditions, flies bearing this factor have a selective advantage over the wild type, suggestive of the possible existence of this mutant in a balanced polymorphic state in natural populations.—No gross external morphological traits are associated with *fs-adp*, but the abnormal fat body of homozygous adults shows a tendency toward hypertrophy. Homozygous males are fertile, females are completely sterile. Eggs laid by mutant females never develop beyond early cleavage stages. They are characterized by an abnormal cytoplasm, frequently yolk-deficient. Irregular meiotic and/or mitotic figures are found, including asynchrony, multipolar spindles, ploidy, chromosomal fragmentation, elimination and restitution. Abnormal egg production, coupled with hypertrophy of the fat body suggests a disturbance in th gonadotrophic activity of the adult ring gland.—The average water content per fly was found to be reduced in mutant adults, while the percentage of ether soluble lipids, as a function of the dry body weight, is almost doubled. The iodine number of total lipid extracts is lower in *fs-adp* flies than in wild type.—The survival of homozygous and heterozygous mutants under conditions of starvation and desiccation was tested. Homozygotes showed a marked advantage when starved, while the heterozygotes were equal to or slightly superior to the various wild type strains tested. Under conditions of desiccation, the heterozygotes displayed, a selective advantage over both of the homozygous types. (Work supported by a National Science Foundation Predoctoral Fellowship.)

Doudney, C. O., and F. L. Haas, Department of Biology, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas: *Macromolecular synthetic sequence in ultraviolet induced mutation in bacteria.*—Preirradiation growth factor supplementation studies have led to the view that ultraviolet-altered nucleic acid precursors constitute metabolic intermediates in UV-induced mutation. Postirradiation studies of the mutation induction process suggest that the "potential mutation" is temporarily "stabilized" by some process requiring energy and amino acids. Following stabilization the mutation is "fixed" by a process which is closely correlated with RNA synthesis.—A number of antimetabolites have been shown to interfere with the mutation induction process. Chloramphenicol and 6-aza uracil, compounds which block protein or RNA and protein synthesis respectively, promote an immediate and rapid decline in mutation frequency. Loss of ability of these compounds to promote decline in mutation frequency is related to the initial postirradiation doubling of cellular RNA. 5-hydroxyuridine and 6-mercaptopurine, compounds which do not block RNA synthesis, promote a decline in mutation frequency which is not immediate but correlated with RNA synthesis in their presence. Studies of labeled analogue incorporation into RNA and the occurrence of mutation frequency decline will be described. Loss of ability of these compounds to promote decline in mutation frequency is not correlated with RNA synthesis but with the prior amino acid dependent "mutation stabilization" process suggesting that the involvement of these analogues in the metabolic process responsible for mutation stabilization is requisite to their effect in antagonizing mutation induction.—Evidence will be presented that protein and then DNA synthesis follow RNA synthesis in the mutation induction process.—Some evidence has been obtained which suggests the segregation of new mutations in the first postirradiation cell division.

DOWNS, WM. G. JR., MEL BOWLING, and G. B. PENNEBAKER, Tennessee Polytechnic Institute, Cookeville, Tennessee: *Two types of dwarfs in the C57 dwarf strain.*—Breeding records on a substrain of dwarf mice from the Roscoe B. Jackson C57 strain have shown unexpected results. These animals are being used as the basis for studies on genetic differences in response to physiologic stress. This makes these results of particular significance.—SNELL's report in 1929 demonstrated that the gene for dwarfism is a simple recessive. Our over-all ratio is consistent with this conclusion. However, the homozygous recessives in our dwarf colony fall into two distinct groups, nearly evenly divided numerically. One of these groups, in size, weight, anatomical make-up and behavior is similar to dwarfs of this strain reported by SNELL and others. The other group rarely exceeds 5 gms in weight, has a lower homeostatic threshold and does not live over 45 days, but otherwise, in anatomy, facial make-up etc. meets the criteria. None of either group will breed. There is no relationship between these factors and color. Diagnosis of dwarfism can be made at 10–12 days, and the distinction between the two types may be seen readily and immediately, but is unquestionable and "fixed" at 20 days.—Not infrequently, a single litter will contain homozygous normal, heterozygous, and homozygous dwarfs of both types.—Statistical handling of these data indicates that allelic (possibly quantitative) genes for "growth" are present in this strain as well as those for dwarfism, are responsible for the differences between the two types, and influence the adaptive mechanism.

DRAY, SHELDON, and GLENDOWLYN O. YOUNG (Introduced by W. E. HESTON), Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Md.: *Genetic control of two γ-globulin isoantigens in domestic rabbits.*—Recent investigations have shown that components of serum from individual rabbits are antigenic in other rabbits. (J. OUDIN 1956, Compt. rend. 242: 2606, 2489; S. DRAY and G. O. YOUNG 1958, Federation Proc. 17: 510, J. Immunol. 81: 142). Subsequently, it was found that 500 domestic rabbits of several breeds could be grouped into three groups on the basis of two isoantigenically different γ-globulins (S. DRAY and G. O. YOUNG (1959) Science 129, 1023). These isoantigenically different γ-globulins, designated RGG-I and RGG-II, were demonstrated with specific isoprecipitins by agar gel immunochemical methods. Individual rabbits were found to contain either RGG-I, RGG-II, or both RGG-I and RGG-II in their sera but never lacked both γ-globulins. Of 500 rabbit sera tested, there were 24 with RGG-I, 379 with RGG-II, and 97 with both RGG-I and RGG-II.—The purpose of this report is to present the results of testing the progeny of over 50 families of domestic rabbits obtained from all six possible matings of the above three groups for the presence of the γ-globulin isoantigens RGG-I and RGG-II in their sera. The results are consistent with the simplest hypothesis that the isoantigenic specificity of the two different γ-globulins are controlled by a single allelic pair of autosomal genes with both γ-globulin specificities present in the heterozygote. The three genotypes may be designated $\gamma^I/\gamma^I$, $\gamma^{II}/\gamma^{II}$, and $\gamma^I/\gamma^{II}$.

DUBES, G. R., and M. CHAPIN, University of Kansas School of Medicine, Kansas City, Kansas: *Pleiotropy in poliovirus.*—Thermostability was found to be positively correlated with inhibition cystine for the Akron $+^{cr}$, *cr*, $cr^{si}$, and $cr^i$ polioviruses: the cystine-requiring $+^{cr}$ and cystine-nonrequiring *cr* viruses, which are not inhibited by cystine, have low thermostability whereas the slightly inhibited $cr^{si}$ and the strongly inhibited $cr^i$ viruses, neither of which requires cystine, have moderate and high thermostability, respectively.—Seven independent revertants from $cr^i$ to viruses less inhibited by cystine were all found to be less thermostable than $cr^i$ and the degree of loss of thermostability was positively correlated with the degree of genetic loss of the property of being inhibited by cystine.—The thermostable Brunhilde *t* poliovirus (kindly supplied by DRS. DULBECCO and VOGT), which they obtained by selective heating and which has no known history of passage on cystine-deficient cells, was found to be inhibited by cystine in contrast to the uninhibited thermolabile wild type Brunhilde.—It is concluded that the poliovirus genetic element which confers cystine-inhibition also confers thermostability on the virus particle. (This research has been supported by the National Foundation.)

DUVICK, D. N., Pioneer Hi-Bred Corn Co., Johnston, Iowa: *A new fertility restorer genotype in cytoplasmic male-sterile maize.*—Three progenies from the $S_1$ of the backcross: WF9(WF9$^T$ × KY21) have been obtained which when crossed to the male-sterile line WF9$^T$ give all male-sterile progeny but which when crossed to the male-sterile SK2$^T$ give all male-fertile progeny. This is a confirmation of the hypothesis (DUVICK 1956, Genetics 41:544–565) that fertility restoration in T cytoplasm depends upon the simultaneous presence of at least two dominant genes, either of which if present as a homozygous recessive, can cause sterility. Thus the genotypes of the various lines involved herein are presumed to be as follows: SK2–$rf_1rf_1Rf_2Rf_2$; WF9–$rf_1rf_1$ $rf_2rf_2$; KY21–$Rf_1Rf_1Rf_2Rf_2$; Selected $S_1$ line–$Rf_1Rf_1rf_2rf_2$. The fertility restorer genotype of WF9 appears to be unique among corn belt inbred lines, since among approximately 90 non-restorer lines tested by the author, only WF9 and possibly two lines derived from hybrids with WF9 have been found to be recessive for two major fertility restorer genes. All other lines are recessive for a single major gene and in all cases where cross tests have been made the recessive gene was $rf_1$. To date no inbred lines, other than the special selection described above, have been found to be of constitution $Rf_1Rf_1rf_2rf_2$. Ways in which this new genotype can be used to make restored hybrids without detasseling will be discussed.

EMMERLING, M. H., Plant Breeding Dept., Cornell University, Ithaca, N.Y.: *Further analysis of the $R^r$ complex in Zea mays.*—In previous studies the occurrence of oblique crossing over was demonstrated by the detection of the deficiency-crossovers $^{(p)}$(S) and (P)$^{(s)}$, but no evidence has yet been obtained establishing the occurrence of the duplication-crossovers (P)(P)(S) and (P)(S)(S). It is possible to detect the (P)(S)(S) crossover by using distinguishable aleurone color alleles, such as *R* Stippled in compound with *R* Navajo-spot. The recombinant type (S)(S) would be recognizable as a single seed with both the Stippled and Navajo-spot phenotypes. A rather extensive test for recombination of these two pattern *R* alleles was made from the cross ♀♀ *g R*-Stippled *K/G R*-Navajo *k* × ♂♂ *g* $r^g$ *k/g* $r^g$ *k*. Out of a total population of 143,522 seeds examined, a number of cases with the Stippled–Navajo spot phenotype was found. These exceptional seeds proved in all cases to be the result of a cytological aberration; none was caused by a crossover gamete (S)(S). On the basis of previous studies in which it was found that the rate of oblique crossing over was $1.3 \times 10^{-4}$, the expected number of (S)(S) crossovers would be approximately 18. The difference between 0 crossovers observed and 18 expected is statistically significant. One interpretation of this study is that the action of *R* Stippled and *R* Navajo on seed and plant color is not dependent upon structurally distinct genic elements. An alternative explanation is that the (S)(S) crossover is not recognizable as Stippled-Navajo spotted seed when (S) components of homologous chromosomes are adjacent to each other on the same chromosome.

ENGLESBERG, E., and N. L. KILLEEN, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pa.: *Mixed gene loci controlling L-arabinose isomerase and L-ribulokinase activities in Escherichia coli B/r.*—GROSS, KILLEEN, and ENGLESBERG (Bacteriol. Proc. 1959) have shown that seventeen L-arabinose negative mutants (*ara*$^-$) of *Escherichia coli* B/r can be divided into three distinct functional groups (A,B,C) based upon sensitivity or resistance to L-arabinose inhibition, accumulation of large quantities of ribulose, and abortive transduction tests. The order of the *ara*$^-$ mutants was determined by transduction experiments with phage P1, employing three and four factor "reciprocal" crosses between the various *ara*$^-$ mutants, with leucine (*leu*) and threonine (*thr*) independence as nonselective markers. All 17 mutants were found to be closely linked and in a linear array, with each functional group, so defined, corresponding precisely to a closely linked genetic group, with the groups arranged in the order *thr*, *ara* A, *ara* B, *ara* C, *leu*.—Recent quantitative enzymatic analysis of extracts of each of the mutants has revealed the following. Each mutant in group A (4) is deficient in L-arabinose isomerase, an enzyme which catalyzes the conversion of L-arabinose to L-ribulose. All group B mutants (8) are deficient in L-ribulokinase which catalyzes the phosphorylation of L-ribulose to ribulosephosphate. This group is mixed, however, in that different kinase negative mutants have different L-arabinose isomerase activities, varying from 1/10 to four times the activity level of the proto-

troph. There is no obvious relationship between these differences in isomerase activity in the B locus and the order of the various mutants involved. All mutants in group C (5) are deficient in both kinase and isomerase.

FABERGÉ, A. C., Zoology Department, University of Texas, Austin, Texas: *Populations of Oxalis with floral trimorphism.*—The genus Oxalis is one of the four known instances of a pollination system involving trimorphic flowers. The genetics of trimorphism has been investigated in some species of this genus by VON UBISCH and by FISHER and MARTIN. FINNEY (1952, Genetica 26:33–64) has examined theoretically the population equilibria to be expected on the assumption of various conceivable genetic determining systems. In many cases equality of the three types (*isoplethy*) is the equilibrium condition, but systems giving other proportions (*anisoplethy*) can be constructed. While north American trimorphic Oxalis species do not lend themselves easily to such work, counts on populations of two of the species have been made. *O. grandis* in southern Indiana gave: long style 1305, mid style 661, short style 623, while *O. Suksdorfii* in Oregon gave: long 246, mid 453, short 207. Thus in both cases there is strong departure from equality of the three types, though the discrepancy is different in the two species. This is quite unlike another unrelated trimorphic plant, *Lythrum salicaria*, in which approximate equality of the three types is found in populations, and in which the genetic system can readily be shown to be isoplethic. Whether the genetics of trimorphism in the two Oxalis species are intrinsically anisoplethic, or whether the departures from equality are due to the superposition of some disturbance on an isoplethic system is not known.

FALK, R., (Introduced by H. J. MULLER), Department of Zoology, Hebrew University, Jerusalem, and Indiana University, Bloomington, Ind.: *Delay in joining of X-ray-induced breaks by anoxia in Drosophila melanogaster.*—OSTER's finding that breaks induced in early spermatids of Drosophila rejoin before fertilization made possible an analysis of the effects of anoxia in this material according to the lines successfully followed in plant material by WOLFF *et al.* and by COHN and in Drosophila oocyte material by ABRAHAMSON. Drosophila 48 hours after pupation were irradiated with 800r, immediately after which they were transferred to a nitrogen atmosphere for six or seven hours. Fourteen hours after the first irradiation, a second dose of 800r was given (it being known from OSTER's observations that at this stage most broken ends join within 12 hours). A number of different controls was run simultaneously. The emerging imagoes were crossed so as to detect induced sex-linked lethals, and translocations between the two large autosomes and between the Y chromosome and the large autosomes. Results showed a significant increase in the frequency of autosomal translocations when anoxia was given for seven hours between irradiations. No effect, or only an insignificant one, was achieved by six hours of anoxia. There is some indication that even nitrogen posttreatment without a second irradiation increased autosomal translocation frequency (see ABRAHAMSON). The nitrogen treatment between irradiations did not appear to increase the frequency of Y-autosome translocations as much as that of interautosome ones, but whether this difference is significant is still being studied. These nitrogen treatments did not significantly augment lethal frequency, although there was a small increase, probably representing lethals connected with gross rearrangements. (Work done while on Postdoctoral Fellowship of U.S. Public Health Service, administered at Indiana University, and supported by grant to H. J. MULLER and associates from U.S. Atomic Energy Commission [Contract AT(11-1)-195]).

FARNSWORTH, MARJORIE W., University of Buffalo, Buffalo, N.Y.: *Studies on isolated mitochondria of Minute and wild type Drosophila.*—Methodology for the isolation of mitochondria from larval stages as well as from adults has been completed. Highly active preparations for spectrophotometric studies can be obtained from as few as 100 to 150 adult thoraces or third instar larvae. For earlier developmental stages, proportionately larger numbers are needed. Preliminary studies indicate that the activities of certain enzymes of the Krebs cycle (e.g., fumarase) do not change during development and are alike in both wild type and Minute larvae. Studies

of other enzymes, including those involved in terminal oxidation, are discussed. (Supported by grant no. C-3286, National Institutes of Health.)

FORSTHOEFEL, PAULINUS F., University of Detroit, Detroit, Mich.: *Anatomy and development of the Springville luxoid mouse.*—Many of STRONG and HARDY's Springville luxoid mice show, on the genetic background of origin, incomplete duplication of the radius and preaxial duplication of the forefoot. Usually the distal portion of the tibia is missing; sometimes, besides the proximal vestige of the one tibia, another more complete slender tibia is also present. In these cases of duplication of the tibia, the preaxial bones of the hind foot are also duplicated.—Breeding data support the hypothesis that the Springville hemimelic luxoid mice are homozygous for a gene which in heterozygous condition causes only preaxial polydactyly of the hind feet. In heterozygous condition this gene interacts with GREEN's luxoid or CARTER's luxate gene in heterozygous condition to give tibial hemimelia.—Homozygous Springville luxoid embryos can be recognized at 11½ days of gestation by incipient polydactyly of the forefeet. At 12 days, the hind feet also show polydactyly and incipient hydrocephaly can be detected by a slight anterior bulging of the telencephalon. By 13½ days the mesencephalon also shows pronounced bulging. The eyes slant back toward the mesencephalon, perhaps because of tension exerted by the outward-pushing mesencephalon. This tension is probably the cause of the failure of the eyelids to close later. At 15½ days, when hair follicles appear on the top of the head of normal sibs, none appear there in the hemimelic mice, apparently the origin of the later baldness. (Supported by grant C-3613 from the National Institutes of Health.)

FOX, ALLEN S., JEAN B. BURNETT, and MORTON S. FUCHS, Michigan State University, East Lansing, Mich.: *Electrophoretically separable tyrosinases of diverse thermostabilities and their interconversion in Neurospora crassa.*—Continuous paper electrophoresis separates the tyrosinase extracted from homocaryons into three components ($T_1$, $T_2$, $T_3$). Since the Michaelis constants and relative turnover numbers (dopa/tyrosine) of the three components are the same, they exhibit indistinguishable specificity. The activation energies for the thermal degradation of the enzymes differ markedly. For $T_1$, $E = 95{,}800$ cal./mole, virtually identical with the value reported by HOROWITZ and FLING for their thermolabile tyrosinase. For $T_2$ and $T_3$, $E = 60{,}900$ cal./mole and 57,300 cal./mole respectively, closely similar to the value for the thermostabile enzyme of HOROWITZ and FLING. In our material, $T_2$ and $T_3$ constitute 96% of the tyrosinase in mycelial extracts: the presence of $T_1$ cannot be detected without prior fractionation by continuous paper electrophoresis. Moreover, during incubation of crude extracts at low temperatures (5°–25°), $T_1$ and $T_2$ disappear. The quantitative decrease in $T_1$ and $T_2$ is exactly compensated by an increase in $T_3$, as if both of the former are converted into the latter. Taken together with our previous demonstration of the first-order activation of protyrosinase by activating enzyme, the terminal stages of tyrosinase synthesis seem to be as follows:

$$P \longrightarrow (T_1 \longrightarrow T_2) \longrightarrow T_3 \qquad \text{or} \qquad \begin{matrix} & & & E_1 & & \\ P_1 & \longrightarrow & T_1 & & & \searrow \\ & & & & & T_3 \\ & & & E_2 & & \nearrow \\ P_2 & \longrightarrow & T_2 & & & \end{matrix}$$

(P, protyrosinase; E, activating enzyme(s); $T_1$, "thermolabile" tyrosinase; $T_2$ and $T_3$, "thermostabile" tyrosinase). The conversion of $T_1$ and $T_2$ to $T_3$ may also be enzymatically catalyzed.—The similarity in specificity and the differences in thermostability and electrophoretic mobility of $T_1$, $T_2$, and $T_3$ suggest differences in tertiary structure rather than in active centers. Specificity is probably determined in earlier stages of synthesis, i.e., at the time of determination of amino acid sequence or during preliminary folding. Genetic control of the rate of conversion, rather than a template mechanism, seems responsible for the preponderance of one or another of the three forms of the enzyme. (Supported by grant no. C-2440, National Institutes of Health.)

FRYE, SARA H., Saint Louis University, St. Louis, Mo.: *Further investigation concerning the frequency and analysis of minute rearrangements of the yellow region in relation to X-ray dose in Drosophila melanogaster.*—Males (0–24 hrs. old) containing the scute-8 Bar chromosome were given 500r or 3000r and these and simultaneous controls were mated to *y w In49 f* virgin-females (48–72 hrs. old). "Yellow mutations," usually representing small deficiencies or inversions, were recorded. Frequencies of exceptional yellows among the $F_1$ Bar females were for the control, 500r, and 3000r, respectively: 0.0022% (2/87,715), 0.0372% (39/98,910), 0.2090% (59/27,925). These and previous comparable results (FRYE 1957) show that yellow mutations, like point mutations and unlike gross rearrangements, vary linearly with dose and not as a power of the dose higher than one.—The yellow region in the present case proves to contain at least the following genes (but not *l46+* (Lüning) or *l259+* (Lindsley) which are allelic to *l71+*) in the sequence: lethal *l1+* (*l71+*), yellow+ (*y+*), achaete+ (*ac+*), bobbed+ (*bb+*). Following the previous symbolism (FRYE 1958) the most frequent class is — — — +, and there are again as well cases of all other types. All — — (±)(±) cases are being tested for the presence of the right end of the II or IV chromosome.—There were two spontaneous minute rearrangements, one was — — — —, the other was — — — — *l*, unlike what was found previously. Neither contained a $Y^S$ (short arm of the Y) on the distal end of the scute-8 Bar chromosome.—The frequency of exceptional yellow[L] males (all sterile), among the $F_1$ *y w f* males, representing gross deletions of the X were, for the controls, 500r and 3000r, respectively: 0% (0/74,017), 0.0147% (12/81,293), 0.1365% (29/21,238). These results show, as expected, that yellow+ exceptions, like gross rearrangements and unlike point mutations, do vary as a power of the dose higher than one. (This work was supported by a grant to I. H. HERSKOWITZ and Associates from the U.S. Atomic Energy Commission [Contract AT(11–1)–633]).

FUNG, SUI-TONG, and JOHN W. GOWEN, Iowa State College, Ames, Iowa: *Sex development in intersexes of Drosophila melanogaster.*—For some years it has been noticed that our triploid stock carrying attached-X's characteristically produces a majority of male type intersexes. A recently isolated triploid stock from a diploid female homozygous for *C3G* (GOWEN and GOWEN 1922) was found to produce an unusually high percentage of female intersexes. In this stock the X chromosomes are separated from each other. Selections were started in this stock toward a high percentage of male type intersex progeny and toward a high percentage of female type intersex progeny. After some generations of testing, two lines characteristically producing high percentages of these opposite types were obtained. Crosses between them show that the type of intersexes produced was largely determined as a maternally inherited characteristic peculiar to the triploid female used as the mother. The four types of back crosses of the $F_1$'s, however, lead to a majority of male intersexual type backcross progeny. Chromosomal determinations on the different progeny types of the respective male and female intersex lines are in progress and will be reported as they are related to the frequencies of the different types of intersexes. (This work has received assistance from Contract Number AT(11–1) 107 from the Atomic Energy Commission.)

GALINSKY, I., Biochemical Research Foundation, Newark, Delaware: *The development of cytoplasmic bodies in a rat ascites tumor.*—Unusual cytoplasmic bodies have been observed in the cells of an ascites tumor of the Wistar rat. These bodies appear round, dark, and uniformly opaque under phase optics. They vary in size and may reach a diameter equal to the width of the chromosomes. They may be found alone, or in clusters containing up to as many as fifty.—The first day after a rat is inoculated with ascites cells, the bodies in the cytoplasm are generally found to be thinly scattered throughout the cytoplasm which very few clusters present. Until the sixth day after inoculation, when the rat usually dies, there is a great increase in the number of these cytoplasmic bodies. Coinciding with this there is also an increase in the number of clusters per cell. In mitosis, the bodies remain outside the nuclear membrane during prophase and outside the spindle region during metaphase, anaphase, and telophase. They are randomly distributed to the daughter cells and sometimes their distribution may be very unequal.—De-

struction of the ascites cell membrane results in the release of the cytoplasmic bodies into the surrounding fluid where they maintain their shape and appearance and exhibit strong Brownian movement not seen in the cytoplasm.—These cytoplasmic bodies will be discussed in relation to their response to cytochemical staining.

GALL, J. G., University of Minnesota, Minneapolis, Minn.: *Uptake of nucleic acid precursors by oocytes of the newt (Triturus viridescens).*—The ovary of the newt takes up adenine-$C^{14}$, cytidine-$H^3$, and thymidine-$H^3$ when these compounds are injected into the coelomic cavity or are mixed with coelomic fluid and placed directly upon an excised ovary. The localization of the compounds has been studied by autoradiography combined with cytochemical tests for the nucleic acids. Sections of fixed whole ovary and preparations of isolated lampbrush chromosomes have been used. Thymidine is rapidly incorporated into the DNA of some follicle cell nuclei, but does not appear in detectable amounts in the nucleic acids of the oocyte. Both adenine-$C^{14}$ and cytidine-$H^3$ are incorporated into the RNA of the oocyte, particularly in the nucleus. Six hours after administration of the compounds the concentration of the isotope (grains per unit area on the autoradiographs) is highest in the nucleoli, next in the chromosomes, and lowest in the cytoplasm. Azure B staining suggests that the concentration of RNA is approximately equal in nucleoli and cytoplasm of the smaller oocytes, but very much lower in the chromosomes. The results indicate, therefore, a very active RNA in the chromosomes. Autoradiographs of isolated chromosomes labelled with cytidine-$H^3$ show that all of the lateral loops are radioactive. However, some loops are more active than others when measured on the basis of grains per unit area. It is not known whether such variations reflect real differences in specific activity of the RNA or are due simply to differences in RNA concentration from loop to loop. (Supported by funds from the National Science Foundation).

GARBER, E. D., University of Chicago, Chicago, Ill.: *The genus Collinsia.* VIII. *Effect of colchicine on C. heterophylla and C. tinctoria.*—Germinating seed and apical meristems of plants of *C. heterophylla* (2n=14) were immersed in aqueous solutions of colchicine for varying lengths of time; the apical meristems of plants of this species and of a closely related species, *C. tinctoria* (2n=14), were smeared with lanolin containing different concentrations of colchicine. Surviving plants with high pollen abortion were self-pollinated and crossed with control plants. The progenies from *C. heterophylla* displayed diploidy, tetraploidy, aneuploidy (trisomes, occasional tetrasomes), or reciprocal translocations, indicative of breakage of two non-homologous chromosomes. The progenies from *C. tinctoria* included only diploids or tetraploids. These observations suggest that *C. heterophylla* and *C. tinctoria* differ in their response to colchicine. (This work was supported by the National Science Foundation and the Abbott Memorial Fund, University of Chicago.)

GARTLER, S. M., University of Washington, Seattle, Wash.: *Incorporation of DNA by mammalian cells in tissue culture.*—Genetic transformation in bacteria is due to incorporation of extracellular donor DNA by host cells. The possibility of genetic transformation in higher organisms, at least in somatic tissue, must be given consideration. Since incorporation of DNA is a prerequisite to genetic transformation, it appeared worthwhile to investigate the possibility of extra-cellular DNA incorporation in mammalian tissues. The results of experiments utilizing mammalian tissue culture lines and extra-cellular homologous labelled DNA will be discussed in this report.—When labelled DNA is injected into the medium of a growing culture a small amount of the label disappears from the medium. This label is not recoverable by repeated saline washing of the cells, or by incubation with DNAse. The label is only recoverable upon lysing of the cells. Furthermore, if the cells are permitted to continue growing after a brief exposure to DNA, the label is incorporated into the host cell's DNA.—Several lines of evidence indicate that at least a part of this adsorbed label is taken up by the cell in a relatively undegraded form.

GERSTEL, D. U., North Carolina State College, Raleigh, N.C.: *Segregation in raw allopolyploids in Nicotiana.*—Gentical segregation in allopolyploids of the genus Gossypium had previously been found to correspond to the taxonomic affinity of the parent species. Autotetraploid segregation frequencies were generally observed where the parents were very closely related; in contrast, hardly any segregation occurred in amphiploids from parents belonging to different taxonomic sections and still other combinations gave various intermediate ratios. A similar situation has now been encountered in Nicotiana. At present, special attention is being paid to the amphiploids of *N. tabacum* with *N. tomentosiformis* and with *N. otophora* because each of the latter species has been claimed, with some justification, to be more closely related to *N. tabacum* than the other. Testcross segregation ratios in 6x *N. tabacum* × *tomentosiformis*, when the amphiploids were used as females, were as follows for four loci: *Yg* 4.5:1, *Ws* 3.4:1, *Pb* 3.2:1 and *Yb* (linked with *Pb*) 3.1:1. Reciprocal crosses gave similar frequencies. The comparable data from 6x *N. tabacum* × *otophora* as females were: *Yg* 14.5:1, *Pb* 10.0:1, *Yb* (linked with *Pb*) 8.3:1 and *Fs* 5.2:1. Thus, 6x *N. tabacum* × *tomentosiformis* segregated essentially in autopolyploid fashion, whereas, in the other combination some preferential chromosome affinity is suspected. Certain complications were noted (e.g., ratios smaller than obtainable from chromatid segregation in *N. tomentosiformis* combinations) and one may tentatively suggest that nonconjunction, chromosome elimination and possibly differential viabilities affected the results more strongly in Nicotiana than in Gossypium. (Supported in part by Grant No. G-4861 of the National Science Foundation).

GOWEN, JOHN W., and LOREN H. HAVERLAND, Iowa State College, Ames, Iowa, and University of Nevada, Reno, Nevada: *Heterosis as a factor in response of quantitative characters to radiation.* —Mice of five inbred lines and their possible hybrids, including reciprocals, were bred for these experiments. The resulting progenies were irradiated with five different X-ray dosages: 0, 20, 200, 400 and 800r. The mice were mated in pairs within the same radiation dosage and kept for their life spans, data being collected on number, growth and survival of their young. The $F_1$ individuals averaged three times the vigor displayed by the inbred pairs. Mean total lifetime progeny of the inbreds was 35 mice, whereas that for the hybrids of the same inbred lines lines averaged 100 mice. Number of mice born shows a simple exponential decline with increasing radiation dosage for each contrasted genotype, inbreds and hybrids. Mean lethal dose for the inbreds was about 103r whereas that for the hybrids was 164r. This difference may be attributed to difference in physiological vigor of the two groups. The physiological system of the inbreds could be said to be only ⅓ as vigorous as the hybrids. On the other hand, the action of radiation is at least ⅔ as effective on the hybrids as on the inbreds. On this basis there is an inverse relation between physiological fitness and radiation effect. However, the effect is less than expected as judged by the performance of these types in producing young. (This work has received assistance from Contract Number AT(11–1) 107 from the Atomic Energy Commission).

GRAF, G. E., University of Notre Dame, Notre Dame, Indiana.: *Uric acid in Drosophila melanogaster.*—A metabolic relationship of purines and pteridines has frequently been postulated. DANNEEL and ESCHRICH-ZIMMERMANN (1957) found that the genes *bw* and *w* caused the disappearance of uric acid in eight day old imagoes. The disappearance was correlated with the isoxanthopterin content of the flies. In the present investigation uric acid was quantitatively determined at a number of stages in several different mutants.— Uric acid is present in small but not significantly different amounts in 3rd instar larvae of all mutants tested including *bw* and *w*. The concentration rises in prepupae and then increases very rapidly in early pupae. There is little change from mid-pupae until after emergence. This resembles the concentration changes found for isoxanthopterin but females had slightly more uric acid than did males. The mutants tested did not differ significantly from wild type. The earlier findings with eight day imagoes were confirmed. A number of *w* alleles or pseudoalleles were studied and the amounts of isoxanthopterin and uric acid were found to be roughly correlated. Reducing the amounts of red pig-

ments through other loci did not decrease the uric acid. The distribution of uric acid in the bodies was studied.

GRELL, RHODA F., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Effect of chromosome four on segregation of sex chromosomes in Drosophila melanogaster.*—Studies have been continued on the non random assortment of a sex chromosome and a fourth chromosome that is observed under certain conditions in *Drosophila melanogaster* (Grell 1957, these abstracts; Genetics, 1959, in press). It was previously reported that in XXY;T(3;4)86D/T(3;4)86D/$ci^D$ females the Y and the free four segregate to opposite poles with a frequency of 92.1% when the two X's segregate regularly (97.4%). The non randomness is interpreted as a result of an association between the Y and the four that occurs in the absence of available pairing partners. Females have now been constructed to measure the effect of a free four on primary and secondary nondisjunction or structurally heterozygous X chromosomes. The frequency of observed exceptions in females of the following four genotypes, where T symbolizes T(3;4)86D, is: Type 1. +/dl-49;T/T, 1.5%; Type 2. +/dl-49;T/T/$ci^D$, 5.2%; Type 3. +/dl-49/Y; T/T, 51.1%; Type 4. +/dl-49/Y;T/T/$ci^D$, 29.2%. After corrections are made for the lethality of some classes of exceptions, a comparison of Types 1 and 2 shows that free four increases the primary nondisjunction of the X's 3.3 times. Furthermore, in 96.7% of the exceptional progeny, the free four and the two X's were found to segregate to opposite poles. A comparison of types 3 and 4 shows that the free four causes a 33% reduction in secondary nondisjunction. The regular progeny from Type 4 shows a segregation of the Y and the four to opposite poles with a frequency of 91.6%.

GRUN, P., Pennsylvania State University, University Park, Pa.: *Cytoplasmic differentiation in the evolution of isolating barriers in Solanum.*—Internal isolating barriers restricting development of $F_1$ hybrids in crosses between closely related species of Solanum were studied to determine the nature of the first stages in development of barriers. Nine species of the series Tuberosa and Commersoniana were used for this purpose, and found to be readily intercrossable in all combinations. A low, but highly significant reduction in seed set in interseries as compared with intraseries crosses does occur. The establishment of these barriers, in some cases, involved separate mechanisms specific to one or the other sex. Evidence is presented for existence of a total of four independent mechanisms reducing seed set in interseries crossings: Male and female mechanism reducing berry set, and male and female mechanism reducing seed set per berry. The evidence is interpreted as showing the existence of a cytoplasmic interaction resulting in either gametic or zygotic selection. In either case, it seems likely that the hybrid individuals grown were not a random, but a selected sample of combinations of genes of the parent species. (Supported by a grant from the National Science Foundation.)

HANSON, W. D., Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland: *The average length of initial linkage blocks intact after n meiotic divisions within selected mating systems.*—A mating population synthesized by intermating $p$ homozygous parents was considered. Thus, $p$ homologous chromosome types (progenitor linkage blocks) would be present in the first generation for each homologous set. The average length of progenitor linkage blocks intact after $n$ generations of mating involved first the identification of the distribution of chromosome types with respect to the total number of points of genetic recombination which had occurred in $n$ meiotic divisions. This distribution was independent of the mating system. Secondly, the distribution of chromosome types with respect to the total number of effective recombinations (recombinations involving loci heterozygous for progenitor chromosome types) was determined which was a function of the mating system. The average genetic distance, with respect to the true genetic map scale, between any two consecutive points of effective recombinations is the average length of initial linkage block intact after $n$ meiotic divisions within a selected mating system. With $p$ large the exact distribution of chromosome types and the density distribution for linkage block lengths was derived for a random intermating

population. With $p$ small in a random intermating population or with inbreeding an approximate distribution of chromosome types with respect to number of effective recombinations was considered which approached the exact distribution as the number of generations became large. The information was used to evaluate the break up of linkage blocks for selected chromosome map lengths under selected mating systems including selfing and selfing from an intermating population. The derivation of average linkage block lengths, as measured with respect to the true genetic map scale, required only the assumption that the occurrence of any two points of genetic recombination was independent in a probability sense. This assumption would not be critical when the total number of points of recombination from $n$ meiotic divisions was considered since recombinations occurring in different generations would be independent.

HINTON, CLAUDE W., and JOHN C. LUCCHESI, University of Georgia, Athens, Georgia: *Observations of meiotic dicentrics and acentrics in inversion heterozygotes of Drosophila.*—Cytological examination of the first and second meiotic divisions of eggs from free-X and tandem attached-X *Drosophila melanogaster* females heterozygous for $In(1)y^4$ revealed 34% (N=85) figures having neither acentrics nor dicentrics. Such normal figures should represent $E_0 + E_2$-2s rank tetrads and the remaining figures having dicentrics and/or acentrics should represent $E_1 + \frac{3}{4} E_2$ rank tetrads. Calculation of $E_1$ and $E_2$ tetrad frequencies from genetic data collected from the tandem attached-X females gives values greater than indicated by the cytological data. The magnitude of the discrepancy is sufficiently small to be the result of uncertainty either in the calculation of tetrad rank frequencies or cytological classification, or both. (Work supported by U.S. Public Health Service Research Grant C-3000.)

HINTON, TAYLOR, University of California at Los Angeles, Los Angeles, Calif.: *Genotypic expression in Drosophila as determined using a chemically defined medium under aseptic conditions.*—When different genetic strains of Drosophila are raised on chemically defined media, under aseptic conditions, a striking difference in response is noted. The difference in response is reflected in the pattern of growth and, in some cases, in an altered phenotype. Some strains will show a change in phenotype when certain metabolites in the medium are altered.—The relationship of the various growth factors, and their effect on the expression of the genotype, is given particular consideration.

HIROTA, Y., Genetics Department, Stanford University, Stanford, California: *Mutants of the F factor in Escherichia coli K-12.*—A contagious plasmid F which occurs in the wild type strain K-12 confers the capacity to act as ♂ in sexual conjugation on strains of *Escherichia coli* (LEDERBERG, CAVALLI, and LEDERBERG). Recently, ADELBERG and BURNS (these abstracts) found a mutant of F termed F′ which conveys the Hfr character (High frequency of recombination). On the basis of this finding, we have isolated distinctive F′ mutants from old broth cultures of several Hfr strains. The F′ mutants were detected by the conversion of F– strains with which they were incubated. Each F′ mutant controls a characteristic pattern of transfer of loci from the ♂ chromosome in the linkage sequence similar to that of the original Hfr from which it was derived. Like standard F+ the new F′ can be disinfected by acridine treatment (HIROTA and ILJIMA) and passed to other F— strains.—These results suggested that the F′ factor is split off from the Hfr locus and liberated into the cytoplasm. Although free of the chromosomal site, it still determines transfer of the chromosome with high frequency, the point of chromosome breakage, and controls the sequence of oriented transfer of genes of the donor chromosome during conjugation. Its specificity might be from the retention of a chromosome segment of the Hfr parent from which the F′ originates. Double infection as well as mutual exclusion have been observed in combinations of nonidentical F factors. F′ is used in this abstract as a class term for F mutants of which $F^2$ (Adelberg and Burns) is an example. F′ (F-prime) should not be confused with $F^1$ (F-one) as they used to denote the wild type F factor. The notation is being rectified.

HOROWITZ, N. H., M. FLING, H. L. MACLEOD, and N. SUEOKA, California Institute of Technology, Pasadena, Calif.: *Multigenic control of tyrosinase synthesis is Neurospora.*—We have identified three unlinked loci in *Neurospora crassa* which function in the synthesis of tyrosinase. At one of these (the *T* locus) there are four alleles, determining four structurally different forms of the enzyme. The latter are distinguishable on the basis of thermostability and/or electrophoretic differences. Two of the six possible heterocaryons between these alleles have been analyzed at this writing; in both cases the heterocaryon makes a mixture of the parental enzymes with no evidence of interaction between alleles.—The other two loci (*ty-1* and *ty-2*, respectively) are characterized by mutants in which tyrosinase synthesis is more or less completely abolished. These mutants carry one or another of the known alleles at the *T* locus. The latter can be extracted by crossing. The tyrosinaseless mutants are recessive to their normal alleles in heterocaryons; the form of the enzyme made by such heterocaryons is determined by the *T* alleles carried in by the two parents.—Both tyrosinaseless mutants can be *induced* to form tyrosinase by growing them in the presence of any one of a number of aromatic amino acids. The form of the enzyme produced corresponds to the allele at the *T* locus.—The results indicate that tyrosinase synthesis is under multigenic control, but the structure of the enzyme is determined by the *T* locus only. The other two loci have secondary roles, possibly concerned with regulating the appearance and disappearance of tyrosinase during the life cycle. This kind of control may be common for nonessential enzymes. (Supported by the National Science Foundation, The Nutrition Foundation, Inc., and the Atomic Energy Commission, Contract AT(04–3)–41).

HOUSE, VERL L., Ohio State University, Columbus, Ohio: *The interaction of Hairless and engrailed with cubitus-interruptus-dominant and cubitus-interruptus-Wallace in Drosophila melanogaster.*—The degree of interruption of the fourth longitudinal vein ($L_4$) in combinations of Hairless (*H*) and engrailed (*en*) with cubitus-interruptus-Wallace ($ci^W$) and with cubitus-interruptus-dominant ($ci^D$) exceeds that expected on a basis of additive mutant effects in combination. These nonadditive effects are particularly pronounced at 18°C. At this temperature $H/+$ possesses normal venation while $ci^W/+$ and $ci^D/+$ are characterized by mean $L_4$ interruptions of 18% and 48% respectively. These interruptions are increased to 66% in $H/+$; $ci^W/+$ and to 75% in $H/+$; $ci^D/+$. Hairless possesses normal $L_4$ and $L_5$ venation in regions proximal to the posterior cross vein while $H/+$; $ci^W/+$ and $H/+$; $ci^W/ci^W$ develop interruptions in these regions greatly in excess of the deletions characteristic of $ci^W/+$ and $ci^W/ci^W$. The alleles *ci* and $ci^D$ produce a scalloping of the posterior wing margin at low temperatures, these effects being inhibited in the presence of Hairless. Engrailed produces an extra sex comb in the male, a cleft scutellum and venation along the posterior margin of the wing. All of these effects are inhibited in the presence of the *ci* alleles. (Work supported by a grant from the National Science Foundation).

HRUBANT, H. E., Long Beach State College, Long Beach, California: *Analysis of differences in free amino acids in the blood plasma of three inbred strains of the house mouse, Mus musculus.*—Statistically significant differences between genotypes were found in mice of the SEC/2-Gn-*d* strain for glutathione (*DD* high), glycine (*Dd* high), alpha alanine (*DD* high) and valine-norvaline (measured together) (*dd* high). No differences were found between the genotypes in the CBA/Ca-*se* strain. Between the genotypes segregating in the Furless strain, there were significant differences found with respect to glutathione (*fsfs* high), aspartic acid (*fsfs* high), lysine (*Fsfs* high), and isoleucine-leucine (measured together) (*Fsfs* high). Significant differences in the level of arginine, lysine, valine-norvaline, and glutamic acid were found between the sexes within a particular strain. Male and female SEC/2 mice differed in the proportion of arginine in their blood, the males being higher. CBA males differed from the females in their proportion of lysine (females higher than males), and valine-norvaline (males higher than females), while the proportion of glutamic acid in Furless males exceeded that in females by a statistically significant amount. All of these sexual differences disappeared, however, when all

mice of all three strains were grouped according to sex and analyzed together. The strains differed statistically with respect to their relative proportions of glutathione, homocysteine, cystine, taurine, glycine, lysine, arginine, alpha alanine, beta alanine, valine-norvaline, and isoleucine-leucine. Despite the presence of the many significant differences, a fairly standardized metabolic pattern can be generated for each inbred strain of mice.

HUGHES, A. M., G. BECKER, and M. CALVIN, Lawrence Radiation Laboratory, University of California, Berkeley, Calif.: *Effects of deuterium oxide (heavy water) on Drosophila melanogaster.*—Drosophila males grown on media in which 10–30% of the added water was $D_2O$ had an increased sterility when compared to controls grown on normal media. The percentage sterility was dependent on the percent $D_2O$ in the media and on the strain. For example, Canton males grown on 20% $D_2O$ media and mated to Basc females resulted in 37% sterile matings, as compared to 15% sterile matings of untreated males. Females grown on media containing 20% $D_2O$ and mated with normal males produced 10% less offspring than untreated females. In 3499 offspring from treated females, as compared to 3174 offspring from control females, the sex ratio males/females was increased 13%. ($P = .012$) This change in sex ratio suggests chromosome damage due to $D_2O$. A developmental anomaly, characterized by abnormal fusion of the tergites, was observed in 11% of the flies grown on 20% $D_2O$ media, compared to 2% in flies grown on normal media. There is no evidence of increase in recessive lethals or influence on meiotic crossing over in flies grown on $D_2O$ concentrations as high as 20%. Studies of somatic crossing over, nondisjunction in the female, and nondisjunction and chromosome loss in the male (using normal females, attached-X females, and ring X males) are now in progress.

IVES, P. T., P. M. CONKLIN and L. R. BURWELL, Amherst College, Amherst, Mass.: *Radiation effects on different strains of Drosophila melanogaster.*—Tests were made of ♂ mutational and ♂ and ♀ longevity responses to γ radiation using inbred Oregon-R-278 and Samarkand-204-20, their reciprocal heterozygotes and the $F_1$ of ♀♀ from the South Amherst natural population. Sperm released during six days in matings of irradiated ♂ to six new *Basc* ♀♀ daily after 2.5kr showed similar daily mutation frequency patterns. There was, however, more than random heterogeneity within days and between strains, coming chiefly from ♂♂ with the Samarkand X chromosome. Longevity measurements after acute doses of 25, 52.5 and 100kr revealed notable variability in the survival curves of only the control and irradiated natural population $F_1$. There were no striking strain differences in average postirradiation longevity. Both types of Samark-and/Oregon-R ♀♀ were moderately heterotic with respect to longevity in all tests compared to the homozygotes. Oregon-R-278 control longevity was much reduced compared to 128 generations earlier and to the other strains tested here. This appears to be due to a deleterious non-visible mutation which also reduced the control longevity of ♂♂ from Oregon-R-278 ♀♀ crossed to Samarkand ♂♂. Differences between strains and combinations with respect to average longevity were proportionately greater in controls than after irradiation so that we have been unable to compute satisfactory LD-50 values.—Both mutational and longevity responses to radiation appear to be subject to genetic modification in phenotypically normal Drosophila.

JAAP, R. G., R. W. TEMPLE, and M. W. MURRAY, Ohio Agricultural Experiment Station, Columbus, Ohio: *Nonadditive gene action on specific body tissues in the young chicken.*—The genetic portions of the variance in weight of the comb, testes, spleen and bursa of Fabricius were high (0.7 to 0.9) in young chickens of the White Gold Randombred strain. Of the total genetic variance at hatching, nonadditive genetic effects accounted for approximately two thirds for comb, one half for bursa, and one third for testes weights. Genetic effects on spleen weight were mostly additive at hatching. At eleven days after hatching nonadditive genetic effects increased, accounting for most of the genetic effects on the comb, and for slightly more than one half on the testes and spleen. Nonadditive genetic effects on the bursa were about the same at both ages. Hormonal response of the comb and testes, prior to 11 days of age, appeared to be due to additive genetic effects. Testosterone stimulation of the comb reduced the nonadditive effects

from two thirds to less than one quarter of the total genetic variance. The P.M.S. gonadotrophin response of the testes, as well as the secondary comb response, was due to additive genetic effects.

JAMES, A. P., Atomic Energy of Canada Ltd., Chalk River, Ontario: *Dominance and overdominance in radiation-induced mutations affecting a quantitative character in yeast.*—A sample of X-ray-induced and spontaneous mutations affecting rate of cell division were assayed for dominance and overdominance. The mutations varied in type from recessive lethal to those reducing rate of cell division in the haploid by as little as 3.5%. The mean growth rate of the heterozygotes for 29 recessive lethals or near-lethals was 8% lower than that of nonmutant diploid controls. A statistically significant reduction in rate of cell division was detected in 19 of these heterozygotes (reductions ranging from more than 30 to less than 2%). A significant increase (1%) in rate of cell division was detected in one heterozygote. The mean rate of cell division of heterozygotes for 29 negative but nonlethal mutations was 2% lower than that of controls. Significant reductions (ranging from 12 to less than 2%) in rate were detected in 13 of the heterozygotes and significant increases (2 and 3%) were detected in two of the heterozygotes.

JANICK, JULES, and J. R. ELLIS, Purdue University, Lafayette, Ind.: *Identification of the sex chromosome in Spinacia oleracea.*—The six primary trisomics of spinach ($n = 6$) have been obtained from triploid pistillate (XXX) $\times$ diploid staminate (XY) crosses. These have been named in accordance with distinctive vegetative characteristics *viz. reflex, oxtongue, star, curled, wild* and *savoy*. Staminate plants of each of the six phenotypes were crossed with pistillate diploids to determine which was trisomic for the chromosome containing the sex determining factor. All of the trisomics with the exception of *reflex* produced diploid progeny in a 1 ♂ : 1 ♀ sex ratio whereas the corresponding ratio of the progeny of the reflex cross was 1 ♂ : 2 ♀. This indicates that reflex was trisomic for the sex determining chromosome with the genotype XXY. From cytological studies, each of the morphological trisomics has been associated with one of the six chromosomes. The chromosome associated with the reflex trisomic, the sex chromosome, is the longest chromosome and is characterized by a submedian centromere. No obvious heteromorphism in this chromosome pair was observed in somatic cells. Heteromorphism involving this chromosome has been reported by ARATAJAN (1939) in *S. tetandra* and recently in two cultivated varieties of *S. oleracea* by ZOSCHKE (1956) and DRESSLER (1958). However, their accounts differ markedly with respect to the morphology of the Y chromosome. The results of this study confirm the existence of races which differ with respect to the morphology of the chromosome containing the XY factors. (This work was supported by the National Science Foundation, G4496.)

KALTER, H., The Children's Hospital Research Foundation, Cincinnati, Ohio: *Seasonal variation in frequency of cortisone-induced cleft palate in mice.*—From November, 1953, to March, 1959, 306 primiparous (C57BL/6 $\times$ A/Jax)$F_1$ female mice, crossed to A/Jax males, were given four successive intramuscular injections of 2.5 mg of cortisone acetate beginning 11⅓ days after conception. In most cases the young were removed before term. The mice bore 2281 offspring of which 2268 had examinable palates. Twelve of these had cleft lip with or without cleft palate. Of the remainder, 1012 ($44.86 \pm 1.55\%$) were affected with median, post-alveolar cleft palate alone. —The distribution of cleft palate incidence by month of treatment showed a marked seasonal variation. Preliminary analysis of this situation showed that the frequency was much greater in the winter months, November to April (163 females, 1147 young, $56.36 \pm 2.07\%$ cleft palate) than in the summer months, May to October (143 females, 1109 young, $36.07 \pm 2.11\%$ cleft palate). This difference is statistically significant ($t = 5.49$ , $P \ll .001$).—Maternal conception weight was taken into consideration, since frequency of cortisone-induced cleft palate is associated with this attribute (J. Exptl. Zool. 134:449–468, 1957); but no difference was found between the winter- and summer-treated females (N-A: $23.00 \pm .22$g; M-O: $22.84 \pm .20$g; $P \cong .5$). A litter-size difference was found, however, in the same direction as previously noted (Am. J. Physiol. 185:65–68, 1956), which apparently indicated an inverse association between litter size and

frequency of cleft palate (N-A: $7.15 \pm .14$; M-O: $7.80 \pm .16$; $P < .01$). (Work supported by grants from the National Institute of Arthritis and Metabolic Diseases, U.S.P.H.S., and the National Vitamin Foundation, Inc., N.Y.)

KAPLAN, W. D., and S. OHNO, City of Hope Medical Center, Duarte, Calif.: *The sex vesicle during meiosis in the male of Mus musculus and Rattus norvegicus.*—The X and Y bivalent, during first meiotic prophase, may be seen to lie imbedded within a structure known as the sex vesicle. This structure is made up largely of RNA as shown by treatment of squash preparations with RNAase. Digestion with this enzyme reveals the individual structures of the X and Y within the sex vesicle so that their relationships to each other during first meiotic prophase may be clearly seen. Under these conditions, the X and Y are found to be positively heteropycnotic and associated end to end. Crossing over between them is unlikely except, possibly, between the extremely short second arms proximal to the kinetochore.

KATO, REI and GEORGE YERGANIAN (Introduced by A. TONOMURA), Children's Cancer Research Foundation, Harvard Medical School, Boston, Massachusetts: *Further observations on the sex chromosomes of the Chinese hamster.*—The sex chromosomes display heteropycnosis during mitotic metaphase and the first meiotic stages. Colchicine-pretreated X chromosomes of spermatogonial metaphases measure 5.0 $\mu$ in length: the short arm is 2.0 $\mu$ long and the long arm, consisting *entirely* of block heterochromatin, measures 3.0 $\mu$ in length, with a nucleolar-organizing region (NOR) 2.0 $\mu$ from the centromere. The mitotic Y chromosome measures 4.5 $\mu$. The short arm of the Y chromosome (1.5 $\mu$) exhibits extensive spiralization during prolonged colchicine pretreatment. In contrast, the corresponding short arm of the X chromosome displays an increased diameter of major spirals (1.5 $\mu$), thereby differing with the Y chromosome morphogenetically except for the moment during meiotic prophase when distal halves of the short arms of both chromosomes are seen to associate.—The meiotic prophase sex bivalent is an amorphous body. During meiotic metaphase, the heterochromatic arm of "block heterochromatin" of the X chromosome is greatly elongated, the NOR is "puffed," and the distal 1/3 of heterochromatin consists of enlarged gyres of loosely spiralled strands. This affinity of the distal portions of the short arms is considered as a "Synaptic Position Effect" which leads to the extensive development of the heterochromatin of the X chromosome only. Such an event may be necessary to instigate the pathways leading to spermiogenesis. Contrast this to the supposed normal staining X chromosome bivalent in the female which is said to undergo crossing over. The distal 1/3 of the short arm of the Y chromosome is regarded as an " activator" or "controller," when coupled in the manner described above.

KELLER, EDWARD C., JR., and DONALD F. MITCHELL (Introduced by J. E. WRIGHT, JR.), Pennsylvania State University, University Park, Pa.: *Interchromosomal genotypic balance in inbred lines of Drosophila melanogaster.*—Three inbred lines of *Drosophila melanogaster* were derived from a natural population and tested to determine interchromosomal genic relationships within and between the lines.—Seven quantitative characters were measured to estimate the effects of the various genetic manipulations; developmental rate, gross body dimension, wing length, bristle number (4th and 5th abdominal segments), percent fertility, percent fecundity, and egg laying rate.—X chromosomes from each inbred line were combined with the autosomes of the other two lines. In one series of tests the donor X chromosome was recombined with a X chromosome from the recipient inbred line. In another series there was no recombination (the X chromosome was transferred isogenically into combination with the recipient inbred line). In the third series the X chromosome from the donor inbred line was heterozygous with the recipient inbred X chromosome (in the females).—It was found that the insertion of identical X chromosomes into the different autosomal backgrounds resulted in different mean expressions for all of the characters studied, but the effects were not predictable. These inconsistencies are the result of interchromosomal genic interactions.—In general the findings support those of MATHER in

that the organization of the genotype can be considered on two levels, i.e., within chromosomal "balance" (linkage) and between chromosome "balance" (homologous chromosomes). There is, however, evidence for interchromosomal "balance" among the nonhomologous chromosomes, hence three levels of genotypic organization can be visualized at the present time.

KIKUDOME, GARY Y., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Some aspects of the phenomenon of preferential segregation in maize.*—LONGLEY (Genetics 30: 100–113, 1945) first demonstrated that the abnormal chromosome 10 induces other chromosomes of the complement to segregate preferentially during megasporogenesis whenever one homologue is knobbed and the other is knobless. The literature contains little or no evidence to show whether the size of the knob is an important factor in preferential segregation. Experiments involving chromosome 9 have shown that, in general, the larger the knob the greater is the degree of preferential segregation for the loci associated with that knob. It has also been shown that whenever one homologue of chromosome 9 had a knob larger than that on the other homologue, preferential segregation favors the chromosome with the larger of the two knobs.—The incidence of crossing over in chromosome 9 was increased in the presence of the abnormal chromosome 10, and the amount of increase was found to vary with the size of the knobs on chromosome 9. Thus, in the knobbed-knobless heterozygotes, the increase over the control values was greatest when the largest knob was involved and least when the smallest knob on chromosome 9 was used. In the presence of the abnormal chromosome 10, however, the recombination frequency was increased to about the same level irrespective of knob size. Evidence favors the hypothesis that the abnormal chromosome 10 increases the frequency of crossing over and that preferential segregation is a function of crossing over.

KIMBALL, R. F., NENITA GAITHER, and STELLA M. WILSON, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *The rate of loss of premutational damage after X-irradiation of Paramecium aurelia.*—Earlier studies led to the following hypothesis about X-ray-induced recessive lethal mutations in *Paramecium aurelia.* Some portion (premutational damage) of the initial radiation effect is subject to loss during the period between irradiation and a terminal event at which the mutational process comes to completion. This terminal event is a particular stage in the normal development of the cell for division and is marked, for reasons as yet not understood, by a sharp drop in "sensitivity" to mutation induction. Postirradiation treatments were thought to decrease mutations by delaying the terminal event thus allowing more time for loss. Delay in the terminal event by posttreatment agents has now been measured by studies of the delay in the associated drop in apparent "sensitivity." It has also been shown that loss of premutational damage does in fact occur. The rate of loss is decreased by posttreatment agents such as streptomycin and chloramphenicol, but the delay in the terminal event is still great enough so that the over-all effect is usually a decrease in mutation. The fact that various agents that inhibit syntheses, especially protein synthesis, decrease the rate of loss of premutational damage is more in accord with the hypothesis that there is metabolic repair of damage than that these metabolic processes are a part of the pathway leading from initial damage to mutation.

KRIVSHENKO, JAKOV, Department of Biology, University of Rochester, Rochester, N.Y.: *The divisibility of the nucleolus organizer and the structure of the nucleolus in Drosophila busckii.* —In the salivary gland cells of *D. busckii* the nucleolus is located between the long distal and the short proximal euchromatic elements of the X chromosome. On (or in) the nucleolus a chromatic body is always present which is the visible part of the nucleolus organizer. It represents the heterochromatized part of the chromosome, and sometimes is distinctly discoidal and at other times is a fibrillar or vesicular structure.—During the period from 1949 to 1958, among numerous X-ray induced mutants, six lines were found which had chromosomal aberrations of different complexity; in each of them one of the breaks occurred in the nucleolus organizer.

These lines are: 1. mosaic [#188, T(X:IIR)]; 2. Cell (#321, inv.); 3. Small spots (#545, inv. and dupl.); 4. pin [#753, T(X:IIIL)]; 5. Small spots [#916, T(X:IIIL)]; and 6. $F_3$-lethal [994, T(X:IIR:IIIR)]. The breaks which occurred in the nucleolus organizer are made apparent by the new permanent connections between the nucleolus and regions or parts of chromosomes which otherwise are never found attached to the nucleolus. From the analysis of these connections it was concluded that the nucleolus organizer is divisible at many points along its length. Independently of its size, each fragment of the nucleolus organizer continues to function, but its effectiveness depends directly upon the size of the organizer. The frequency of breaks in the nucleolus organizer is apparently the same as (or at least is not lower than) in other regions of chromosomes.—The results of the study of the connections between the chromosomal elements and the nucleolus in normal and aberrant nuclei of salivary gland cells, using different types of microscopes (light, phase, and polarizing) and different methods of making preparations, allow us to conclude that the nucleolus of these cells represents a structure in which the microscopically visible, as well as the unresolvable, branches of the nucleolus organizer take a direct part.

La Chance, Leo E. (Introduced by L. A. Grodzins), Brookhaven National Laboratory, Upton, N. Y.: *The enhancement of chromosome aberrations by $CO_2$ and its dependence on the linear energy transfer of various radiations.* When Tradescantia inflorescences are X-irradiated in air and compared to those irradiated in air plus $CO_2$, the latter groups show an increase in chromosome aberrations (Proc. Natl. Acad. Sci. U. S. 38: 809, 1952). To determine if this *carbon dioxide effect* is common to other types of radiations, Tradescantia inflorescences were exposed to fission neutrons (energy range from 0.5 to 2.5 Mev) and to monoenergetic 15 Mev neutrons from a D, T reaction. In each experiment two groups of inflorescences were irradiated; one in air and the other in air plus 1 atm of $CO_2$. The inflorescences were fixed four days after irradiation, stained with propiono-carmine and cells in metaphase were scored for dicentrics, centric rings and interstitial deletions. With 300r of 250 KVP X-rays chromosome interchanges increase 132%, and interstitial deletions increase 245% when the treatment is given in air plus $CO_2$. With a 10-minute exposure to 15 Mev neutrons (estimated 100 rads) chromosome interchanges increase 17% and interstitial deletions increase 25%, but only the latter difference is highly significant. A two-minute exposure to fission neutrons (estimated 50 rads) gave no significant difference in interchanges or interstitial deletions between groups irradiated in air or air plus $CO_2$. The results indicate that the carbon dioxide effect appears only with radiations which have a low LET value. Since broken chromosomes can move over short distances to form aberrations, a tentative hypothesis is offered that carbon dioxide promotes the movement of breakage ends and favors rejoining. It is most effective when a large proportion of the breaks result from separate tracks. With radiations of high LET where two breaks result from one track, chromosome movement becomes less important and the carbon dioxide effect disappears. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

Latterell, Richard L.[1] (Introduced by Arnold H. Sparrow), Biology Department, Brookhaven National Laboratory, Upton, N.Y.: *Changes in radiosensitivity of maize chromosomes during seed germination.*[2]—This paper describes changes in radiosensitivity of seeds during germination and attempts to relate these changes to alterations in physiological activity.—Maize seeds heterozygous for alleles of the $yg_2$ locus were irradiated with 800r of X-rays after having been soaked at 27°C for times up to 36 hours. Somatic sectors, representing losses of the dominant allele, provide a criterion of chromosome breakage. Sector frequency increased gradually during the first 16 hours of germination then rose abruptly (10–14 fold) from 16–22 hours after which a plateau of radiosensitivity was reached.—Oxygen uptake was measured for samples of unirradiated seed soaked for comparable periods. Rate of oxygen uptake increased steadily throughout the 36-hour period.—Dry seeds and seeds soaked up to 30 hours were irradiated in atmospheres of air or helium. The presence or absence of oxygen during irradiation of dry seed had no influence on the frequency of somatic sectors. However, a marked reduction in sector fre-

quencies resulted when seeds soaked for six hours or longer were irradiated in helium. The oxygen effect, i.e., increased aberration frequency in the presence of oxygen, was maximal at about six hours and decreased with longer periods of germination.—These findings suggest that the abrupt rise in radiosensitivity (16–22 hours) is related to metabolic changes and essentially independent of the effect of oxygen in enhancing chromosome breakage. Some physiological implications of these studies will be discussed.

LEVITAN, M., Woman's Medical College of Pennsylvania, Philadelphia, Pa.: *A non random association of inversions correlated with their geographic distribution.*—Several intensively studied local populations of the eastern woods species *Drosophila robusta* contain significantly fewer chromosomes combining gene arrangements 2L-3 and 2R-1 than expected on a random basis. The geographic distribution of these arrangements suggests that the selective disadvantage of the combination is widespread, for there tends to be an inverse correlation between the amounts of the two arrangements present.—At altitudes below 500 feet 2L-3 has frequencies of less than 1% below 40° N. latitude. Concomitantly 2R-1 decreases clinally from 50% to 20% as one proceeds northward. In a collection at 41° N. 2L-3 jumped to 25% and 2R-1 dropped to 2%.—Between 500 and 999 feet 2L-3 is between 0 and 11% south of 41° N.; in two populations between 41° and 43° N. it jumps to 30%. At these altitudes 2R-1, which decreases from 27.5% to 10% with increased latitude, is generally nearer 20% where 2L-3 is close to zero, nearer 10% where 2L-3 is higer. North of 43° N. 2L-3 jumps to over 75% and 2R-1 falls below 5%.—Above 1000 feet 2L-3 is 20% or higher, and here 2R-1 is always below 15% (even in the South). At the 43rd parallel 2L-3 approaches 100% and 2R-1 about 5% or less. At these elevations, however, the relation of the two arrangements to latitude and the inverse correlation between the two arrangements are much more irregular. Possibly additional ecological factors, such as the absence of the American elm from certain areas, become important in the higher altitudes. (Supported by a grant from the National Science Foundation.)

LEWIS, E. B., California Institute of Technology, Pasadena, Cal.: *Germinal and somatic reversion of the ivory mutant in Drosophila melanogaster.*—On the basis of attached-X and free X studies, the ivory ($w^i$) mutant lies to the left of the locus of white ($w$) at 1.5 in the X chromosome. Crossing over appears to be significantly lower between $w^i$ and $w$ (or $w^{ch}$) than between $w$ and other pseudoalleles of $w$ such as, apricot, buff, and white-17G2. The pseudoallelic analysis of $w^i$ is also complicated by the occurrence of noncrossover reversions of ivory as well as wild type crossovers of the type expected on the basis that ivory is to the left of $w$ (or $w^{ch}$), the numbers being 21 noncrossover reversions to six wild type crossovers. The frequency of noncrossover reversions in different experiments varied between 6 among about 31,000 to 5 among about 187,000 ivory female gametes recovered. These reversions are probably reverse mutations rather than conversions since homoygous $w^i$ females also show a relatively high frequency of reversion (15 among about 290,000 ivory female gametes recovered). Reversion of $w^i$ occurs much more rarely in the male germ line. X-rays markedly increase the frequency of reversion of $w^i$ in the female germ line (29 cases representing a minimum of 8 or 9 separate occurrences of $w^i$ reversions among about 120,000 ivory female gametes recovered from females receiving about 4,000r). Only 16 reversions occurred, and these as a single cluster, among approximately 125,000 ivory male gametes recovered from males receiving 3,000r. Somatically, reversions occur spontaneously, though rarely, in $w^i$ males and homozygous $w^i$ or $w/w^i$ females, and are increased significantly in such individuals following X-ray treatment in early larval stages. Frequency of somatic reversion of ivory provides a convenient index for measuring mutagenesis in Drosophila. (Work supported in part by a grant from AEC—At-04-3-41.)

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[1] Public Health Service Research Fellow of the National Cancer Institute.

[2] Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

LEWIS, H. W., and H. S. LEWIS, Massachusetts Institute of Technology, Cambridge, Mass.: *Quantitative aspects of the genetic control of tyrosinase activity in Drosophila melanogaster.*—A survey of 40 strains of *Drosophila melanogaster* has shown that the level of tyrosinase activity in crude extracts varies over a wide range. These variations are not correlated with any phenotype associated with pigment formation. Mixing experiments with homogenates has shown that the reduced level of enzyme activity in the low enzyme lines is not due to the presence of an inhibitor. The nature of the genetic control of this enzyme system was examined by a series of crosses between low enzyme activity and high enzyme activity strains. In crosses involving Canton-S as the low enzyme level parental strain and a sable garnet ($s\ g^3$) strain as the high enzyme level parent the tyrosinase activity of the $F_1$ approached the geometric mean of the parental activities. The enzyme activity of the progeny of subsequent backcrosses to the $s\ g^3$ strain likewise approached the geometric means of the parental values in each backcross generation. This indicates that the level of tyrosinase activity is controlled by a large number of loci and therefore considerable variation in tyrosinase activity should be expected among the individuals of a particular stock. Intrastrain heterogeneity for enzyme activity was measured using the Samarkand wild type strain. The progeny of single pair matings were assayed and a selection program carried out for a high enzyme level and a low enzyme level line. Such lines were readily established. Crosses between Samarkand and pink-peach ($p^p$) as the high and low parental strains respectively revealed strikingly different genetic control of tyrosinase activity. In this case the mean level of enzymatic activity of the $F_1$ population was significantly higher than the arithmetic mean of the parental strains.

LIEB, M., Brandeis University, Waltham, Mass.: *Factors influencing the postirradiation appearance of mutants in Escherichia coli.*—Doses of UV light that produce little or no killing have been used to induce *Try+* mutations in a tryptophane requiring strain of *Escherichia coli* B/r. The frequency of mutants that can be recovered is reduced if protein and DNA synthesis are suppressed immediately after UV. Incubation in a complete medium + chlorampenicol, in a medium lacking tryptophane, or in saline are all effective methods of reducing mutation. Selection can be excluded as an important factor in the decline in mutant frequency.—The great majority of mutants capable of growing without tryptophane appear only after the irradiated bacteria have divided 1–2 times. The appearance of mutant bacteria can be correlated with synthesis of DNA and protein, rather than of RNA. O-point mutations seldom account for more than 10% of all *Try+* mutants, and are relatively less frequent after UV doses that produce appreciable killing. UV-irradiation of logarithmically-growing cultures produces few if any O-point mutations. X-ray studies suggest that the percentage of mutations that appear without intermediate cultivation is not directly related to the amount of killing produced by a given dose of radiation, but to its effect on mutation and nucleic acid synthesis. These findings will be discussed in relation to current theories of DNA replication and mutation.

MA, TE-HSIU, (introduced by W. R. SINGLETON), Blandy Experimental Farm, University of Virginia, Charlottesville, Va. *Cytogenetic studies of gamma radiation of maize.*—The ontogenetic development of *Zea mays*, L. was studied in the green house and the field. Ten ontogenetic phases were determined by morphological features. These covered the period from anther formation, microsporegenesis and sexual cell differentiation. Four cytological phases within this period were used for the study of effectiveness of induction of chromosome aberration and genetic change. These are pre-meiotic, meiotic, pollen mitotic and mature pollen respectively. These four cytological phases can be delimited by the morphological features of the ontogenetic phases.—Eleven hundred r of gamma irradiation from a cobalt-60 source was applied in a twenty-three and half hour period to the dominant tester plants at the early stages of each of the four cytological phases. Chromosome aberrations were determined from the material fixed at the cytological phases following the phase to which the treatment was applied, and from the fixations made immediately after treatment as well as from the fixations of $F_1$ plants. Mutations concerned in this investigation were confined to the endosperm characters and aleurone color

of the corn kernel. Genetic study was done by pollinating dominant tester stock to the untreated recessive tester stock. Gross mutants were scored from the resulting ears, pseudomutants and true mutants were differentiated by raising the gross mutant seeds and analyzing the ears which resulted from the selfed $F_1$ plants. Pollen mitotic phase was found to be the most effective period for the induction of chromosome aberration and genetic change.—A high rate of fragment-free bridges was scored in the $F_1$ population in addition to the expected transmitted aberrants. Hetero-fertilization played an important role in the genetic events. Restitution and rejoining of broken chromosomes as well as the screening out of fragments through cell division were apparent.

McDONALD, D. J., Department of Biology, Dickinson College, Carlisle, Pa.: *Sex-linked selection in Tribolium confusum.*—The mutation Striped ("St") is a recessive sex-linked lethal of *Tribolium confusum.* Heterozygous females have white stripes on their elytra while the elytra of surviving homozygous females and hemizygous males are entirely white. Since not more than 10% of the two latter types survive and those that do are rarely fertile, selection against them is nearly complete. Therefore, in growing populations, in each generation the frequency of heterozygous females should be reduced to one half its value in the preceding generation. Data from large populations of heterozygous and wild type individuals, propagated by using 1000 offspring from the previous generation to start the next, indicates that the frequency of heterozygous female declines more rapidly than predicted. However, since crosses of heterozygous females with wild males yield only 78% of the expected number of heterozygous female offspring, adjusting the theoretical selection curve by including this partial selection against heterozygous females improves the fit of the theoretical to the experimental curves.—In population cages additional factors may influence the course of selection again "*St*". If offspring are collected as they emerge the ratio of wild females to heterozygous females declines from a value of 1.8 to about 1.1, indicating a later eclosion for the heterozygous females. There is also evidence of fertility and longevity differences between these two types of females. (Research supported by NSF Grant G—5488 and USPHS Grant RG—5406.)

MACRAE, EDITH KRUGELIS, and IRA M. ROSENTHAL, University of Illinois, College of Medicine, Chicago, Ill.: *Enzyme studies of hereditary acute and congenital porphyrias.*—Acute porphyria and congenital porphyria in the human organism have been described as disturbances of porphyrin metabolism. Acute porphyria (a Mendelian dominant) is characterized by urinary excretion of porphobilinogen (PBG), an intermediate of porphyrin metabolism. Congenital porphyria (a Mendelian recessive) is characterized by urinary excretion of uroporphyrin (U) and coproporhyrin (C). Hemolysates of red blood cells (RBC) from both porphyria types were tested and compared with those of normal controls with regard to enzymatic conversion of δ-amino levulinic acid (ALA) to porphobilinogen (PBG) and PBG to uroporphyrin (U). In the case of acute porphyria (5 cases), RBC hemolysate studies do not indicate any enzymatic block in the conversion of ALA to PBG or PBG to U. The genetic disturbance probably resides in steps prior to the formation of PBG rather than in utilization of PBG. In the case of congenital porphyria (1 case), RBC hemolysate studies indicate a conversion of ALA to PBG and PBG to U similar to normal controls. However, PBG is converted to the isomer I of U as well as to the isomer U III. In normal RBC hemolysates only U III is formed. This study indicates that the genetic disturbance may reside in the conversion of PBG to U III. These latter results are in agreement with those of RIMINGTON and BOOLJ (1957) and GRANICK and MAUZERALL (1958). (Work supported in part by a University of Illinois Research Grant.)

MAGUIRE, MARJORIE P., University of Texas, Austin, Texas.: *A study of homology between a terminal portion of Zea chromosome 2 and a chromosome segment derived from Tripsacum.*—In the course of studies on cytogenetics of *Zea mays* hyperploid for chromosomes derived from *Tripsacum dactyloides* a $2N + 1$ stock was isolated containing a pair of Zea-Tripsacum interchange chromosomes. One of these chromosomes carried a terminal portion of Zea chromosome

2S attached to a segment of a Tripsacum chromosome bearing no apparent homology to any Zea chromosome. The reciprocal exchange contained the remainder of Zea chromosome 2 including its centromere and a portion of the Tripsacum chromosome marked by a terminal knob equal in length to the distal segment of Zea chromosome 2S for which it had been exchanged. Genetic tests indicated that this latter Tripsacum segment carried "*Lg*" and "*Gl*" genes capable of dominating "$lg_1$" and "$gl_2$" genes of Zea chromosomes 2S, although recombination between Zea chromosome 2 and Tripsacum segment in this region was very rare. At pachytene in disomic plants carrying one normal Zea chromosome 2 and one exchange ZT chromosome 2, the corresponding Zea and Tripsacum segments were commonly paired throughout, although pairing failure, usually terminal, occurred with varying frequency and extent in this region. In studies of more than 8,000 clear cells similar pairing failure was observed to a lesser degree in pachytene bivalents of each of the other chromosomes. The distributions, frequencies and extents of these failures were studied where possible and certain correlations noted, such as a direct relationship of relative chromosome length to failure frequency. The findings are discussed in relation to the concepts of chromosome homology and synapsis.

MALING, BARBARA, Department of Biological Sciences, Stanford University, Stanford, California: *The alteration of crossing over in Neurospora crassa by analogues by thymine and phenylalanine.*—A variety of agents were screened for their effect on crossing over by measuring the frequency of prototrophic progeny from a cross between linked nutritional mutants. The most effective treatments were tested further by analyzing ordered asci (350 to 500 for the control and each treatment) from a cross in which crossing over could be detected in five intervals in two linkage groups. Nutritional requirements were determined by replica plating. To obtain accurate estimates of exchange frequencies in gene-centromere intervals, correction was made for ascospore rearrangement which could be attributed to partial overlap of second division spindles. The frequency of partial overlap was significantly increased by the treatments.—p-fluorophenylalanine causes a significant increase in crossing over near a centromere and 5-bromouracil causes significant decreases in mid-arm intervals. Positive chiasma interference exists in both linkage groups, but is stronger in one group than in the other. Chiasma interference is not affected by treatment. Negative chromatid interference is highly significant in crosses treated with 5-bromouracil.—It is possible that 5-bromouracil and p-fluorophenylalanine act by producing abnormal structural components in the chromosomes. However, even if this hypothesis were correct, these analogues need not be directly involved in crossing over. The fact that these agents elicit different responses in different regions of the chromosome indicates that they may not act solely by altering the lability of a particular type of chemical bond distributed throughout the chromosome. (Supported in part by National Science Foundation research grant 3840 and by Public Health Service research and training grants E1462 and 2G-158.)

MARGOLIN, PAUL, Biological Laboratory, Cold Spring Harbor, N. Y.: *Linkage order and complementation among nonidentical alleles of the leucine locus of Salmonella typhimurium.*—Using transduction, the order of the sites of more than 20 spontaneous, independent leucine auxotrophs have been determined. The recombination frequencies were obtained from three point tests involving two independent leucine alleles plus arabinose as an outside, closely linked marker. Reciprocal transductions between two such alleles and comparisons of the ratios of *ara*$^+$ to *ara*$^-$ among the resultant prototrophic recombinants permit a determination of the relative positions of the alleles, although linkage distances cannot be accurately determined.—Transductions are being continued to add more sites to the linkage map, using the more than 100 additional leucine auxotrophs which are available. The alleles are also being tested for their sensitivity to various mutagenic agents with respect to reversion to prototrophy. This is being done to determine if there are regional patterns of response.—All leucine auxotrophic alleles tested thus far were found to fall into four complementation groups as determined by the formation of minute colonies indicative of abortive transductions. Each allele is capable of complementing all other alleles except those within its group. Thus far each group is linearly homogenous. Tests of a sample of

alleles from the four complementation groups indicate that no feeding occurs between auxotrophs, whether of the same or of different groups.

MARTIN, ALBERT, JR., Veterans Administration Neuropsychiatric Hospital, Pittsburgh 6, Pa.: *Varieties I, II, and III in the species Vibrio tyrogenus.*—Tap water, after passage through a Selas 03 filter, yields a luxuriant growth of *Vibrio tyrogenus* when inoculated into two percent tryptone broth and incubated for 1–4 weeks at 30°C. Three distinct varieties of this species may be differentiated on the basis of culture characteristics and slide agglutination tests. The chief criteria for the establishment of three varieties are as follows: Variety I, light general turbidity with little or no discoloration of two percent tryptone broth nor rabbit blood agar: variety II, marked turbidity with amber coloration of two percent tryptone broth, and an olive-green discoloration of rabbit blood agar; and variety III, heavy surface pellicle, general browning of two percent tryptone broth, and little or no discoloration of rabbit blood agar. Human sera tested for antibody content shows a marked three-plus agglutination using variety II as the antigen, and variations from one to three-plus when variety I or III is employed as the antigen. The protein of both varieties is sufficiently similar, however, to elicit a positive reaction with 98 percent of all sera tested, and does thereby attest to a common genotype for this fresh-water free-living saprophyte. Media made by laboratory procedures employing distilled water, even filtered distilled water, may possess either one or all varieties of this species. Particular attention must, therefore, be given to the sterilization of culture media in all microbiological procedures employing fresh, distilled, or filtered water. The phenotypic, and thus genotypic, varieties discernible in this species are of value in following the effect of continued artificial cultivation on bacteria, and the incidence, frequency, and similarity of reverse mutations.

MEGNET, ROLAND (introduced by N. H. GILES), Yale University, New Haven, Conn.: *Complementation between allelic adenylosuccinaseless mutants at the $Ad_8$-locus of Schizosaccharomyces Pombe.*—Of 54 UV-induced, adenine-specific, haploid mutant strains of *Schizosaccharomyces Pombe* str. *liquefaciens*, 24 mutants have been found by genetic tests to be at the *ad-8* locus. These $ad_8$-mutants lack the enzyme adenylosuccinase and are thus similar to *ad-4* mutants of *Neurospora crassa.* To test for possible interallelic complementation at the $ad_8$-locus, an additional gene resulting in a growth factor requirement (either $ur_1$ or $arg_1$) was introduced into each of these mutants. Matings in all possible pairwise combinations have been initiated to produce diploid cells with the genotype $ad_8/ad_8$, $Ur_1/ur_1/Arg_1/arg_1$, $h^+/h^-$. In a certain number of combinations of $ad_8$-alleles, interallelic complementation has been found to occur; i.e., certain combinations of haploid cells give rise to adenine-independent diploid cells. The specific activity of adenylossuccinase in these complementing heterozygous diploids does not exceed 25% of the activity in the wild type.

MERICLE, L. W., and RAE PHELPS MERICLE, Michigan State University, East Lansing, Michigan: *Changes in amino acid content induced by the X-radiation of developing barley embryos.*—Barley embryos (Hannchen variety) within developing heads of plants were exposed to a single acute dose of 400r X-rays at specific stages of embryogeny. Control and irradiated embryos were permitted to remain on the plants until differentiation was complete, then were dissected from the grains and hydrolysed in HCl. Single and two-dimensional paper chromatograms of the amino acids were prepared from the hydrolysates. Although 18 amino acids were qualitatively identified in the control materials, a few of them were not resolved sufficiently to permit individual quantitation. Three of the six irradiated samples showed depression of total amino acid content in the hydrolysate on the basis of dry weight; none showed an increase. In all the irradiated samples, certain amino acids assumed new quantitative relationships to one another, usually as a result of their differential depression. Among the more marked changes, proline, threnonine, valine, and cystine/cysteine were depressed in some instances to the extent of nondetection. Glutamic acid, on the other hand, was most commonly increased to levels above that of the controls. Alanine and tyrosine showed the greatest fluctuations varying from a marked

depression (with tyrosine sometimes not being detected), to no depression below control levels even when the total amino acid content was markedly down, while at other times higher than control levels (with tyrosine in one instance amounting to twice that found in the controls). These results show that X-radiation may have not only an over-all depressing effect on total amino acid content, but also a differential effect on the individual amino acids. None of the amino acid differences, however, either collectively or individually appeared to be stage specific or radiation specific. (Supported by the Atomic Energy Commission.)

MERRELL, DAVID J., University of Minnesota, Minneapolis, Minn.: *Heterosis in DDT resistant and susceptible populations of Drosophila melanogaster.*— Two DDT resistant populations, each approximately 100 times as resistant as its control, were crossed and the $F_1$ and $F_2$ hybrids tested for fecundity, fertility, and DDT resistance. Similar crosses and tests were made with the controls. Heterosis was observed in the resistant $F_1$ hybrids for all three traits, but only for fecundity in the $F_1$ control hybrids. $F_2$ values were generally somewhat lower than the $F_1$, but there was no indication of hybrid breakdown in the $F_2$. Since each of the four originally heterozygous populations had been maintained in isolation for six years, evidence was sought for the development of coadapted genetic systems during this period, but no indications of coadaptation were found.

MERZ, TIMOTHY, NORMAN S. COHN and CARL P. SWANSON, Department of Biology, Johns Hopkins University, Baltimore, Maryland: *The interaction of localized and randomized chromatid breaks produced by treatment with X-rays and radiomimetic compounds.*—It has been demonstrated that compounds such as 8-ethoxycaffeine and maleic hydrazide affect specific regions of the broad bean chromosomes resulting in localized chromatid breaks. Two types of experiment were used to determine whether breaks induced by one agent would interact with those produced by treatment with another. The first is used with agents which produce breakage frequencies undiminished in the presence of metabolic inhibitors. Since these inhibitors (such as DNP) prevent restitution, all of the breaks induced by one agent should be available for interaction with the breaks produced by another. Using this method, the interaction of breaks resulting from KCN treatments (localization in the heterochromatic regions of the chromosomes) and X-ray treatments (randomized breakage) were tested; exchange frequencies indicated that complete interaction had taken place between the breaks. The second type of experiment is used when breakage frequencies produced by treatment with one or both agents are diminished in the presence of metabolic inhibitors and treatment by both agents results in characteristic breakage in specific regions of the chromosome. Such is the case with 8-EOC and MH. Exchanges between breaks at different specific localities indicated interaction, and some qualitative similarity, whereas lack of interaction indicates, qualitative differences. An analysis of this kind of interaction or lack thereof leads to information regarding chromosome structure particularly at specific breakage points. (Supported in part by The National Institutes of Health, The National Science Foundation and The U.S. Atomic Energy Commission.)

MEYER, HELEN U., ELIZABETH F. EHRLICH and H. J. MULLER, Indiana University, Bloomington, Ind.: *Tolerance of gonial cells of Drosophila melanogaster for heavy X-ray doses divided into installments.*—To explore ways of obtaining offspring from heavily irradiated gonia, X-ray installments of 1500r, 3000r, and 4000r were given to young imagoes. To promote gonial proliferation, installments were separated by 2-, 4-, 5- or 8-day intervals, during which the flies fed and reproduced actively. Adult offspring from eggs laid in successive broods after the last irradiation were counted. After female irradiation, only the first two 4-day broods (from treated oocytes) were strongly affected by size of installment. The earliest broods representing treated gonia (brood 3 from treated females, broods 4 and 5 from treated males) gave the highest yields. Four-day intervals gave distinctly higher yields than 2-day, but not much lower than 8-day intervals. Tolerance varied greatly with genotype. Thus, with 4000r installments five days apart, 8000r sterilized all oogonia of a homozygous stock marked only by veinlet, while 24,000r allowed some oogonia of normals from outcrosses to reproduce. With similarly

given installments, 12,000r sterilized all spermatogonia of yellows while 24,000r allowed some spermatogonia of Stubbles to reproduce. In experiments ranging from 6,000r to 24,000r, the yield of offspring from treated oogonia was approximately halved by each 3000r given (as contrasted with 2000r for the halving dose earlier found when late spermatozoa are irradiated in males). The following sex-linked lethal frequencies were induced in oogonia in the last-mentioned series: 12,000r (3000r installments), 13/244; 16,000r (4000r installments), 15/175; 24,000r (4000r installments), 19/125; that is, 1% per 2000r. Crossover tests showed these very rarely to include gross structural changes. (Work supported by a grant from the U.S. Public Health Service, Grant No. RG 5286 (C1).)

MORSE, M. L., University of Colorado Medical Center, Denver 22, Colo.: *Recombination and segregation in Gal heterogenotes showing position effect.*—The $Gal_1^-$, $Gal_4^-$, $Gal_6^-$, $Gal_7^-$ mutants of *E. coli* K-12 fail to produce an active *Gal*-P-transferase enzyme. These mutations are separable in crosses, the frequency of $Gal^+$ prototrophs being $0.1–3.0 \times 10^{-4}$. Previous analysis by lambda bacteriophage transduction of $Gal_1$, $Gal_4$ heterogenotes indicated that they were members of a cistron, with mutant phenotype (−) in *trans*-positions(+−/−+, −+/+−) and wild type (+) in *cis*-positions(−−/++, ++/−−). Heterogenotes have been made between all members of this group. Excepting the $Gal_4$, $Gal_7$ pairs, heterogenotes have: (1) a mutant phenotype in the *trans*-position; (2) wild type phenotype in the *cis*-position. Both $Gal_4$, $Gal_7$ *trans*–position heterogenotes have a (+) or intermediate (+) phenotype and both *cis*–position heterogenotes are (+). The anomalous behavior of $Gal_1$, $Gal_7$ violates the cistron concept. Similar observations have been made by PONTECORVO (adenine mutants of Aspergillus) and probably by E. LEDERBERG ($Gal_2$, $Gal_9$ mutants of *E. coli*). The (−) phenotypic *trans*-position (+−/−+, −+/+−) heterogenotes segregate *Gal*⁻ and in addition undergo automictic recombination to yield (+). The probability per division of segregation from the *trans*-position is $1–3 \times 10^{-3}$, not significantly different from the probability in nonposition effect heterogenotes. The probability per division of automixis between $Gal_1$, $Gal_4$, $Gal_6$, $Gal_7$ (excepting $Gal_4$, $Gal_7$) to give (+) is $0.3 – 5.0 \times 10^{-4}$, ten-fold lower than the segregation probability, and not significantly different from the meiotic crossing over rate.

MUNDKUR, BALAJI, Department of Anatomy, University of Chicago, Chicago 37, Ill.: *The fine structure of vesicular nuclei in frozen-dried Saccharomyces.*—Cytological work at the light microscope level has revealed that nuclei in frozen-dried preparations cleave by elongation and medial construction without dissolution of the nuclear membrane. They are never differentiated into morphologically detectable chromosomes at any stage of nuclear division but have their DNA in extraordinarily uniform dispersion as submicroscopic particles throughout all stages of division. Nevertheless, interphase nuclei in a polyploid series exhibited progressive volume increments from haploid to tetraploid, as expected. Electron micrographs of sectioned yeast have now been obtained after ultra-rapid freezing and drying, followed by postfixation *in vacuo* with alcohol vapor and/or heat denaturation of cellular protein. Selected micrographs illustrating the appearance of cells following various staining procedures will be shown; these include the use of gallocyanin-chromalum for basophilia, as well as control treatments involving ribonuclease and perchloric acid extraction, alcoholic platinic tetrabromide as a general stain, and lead nitrate for the precipitation of vacuolar inclusions. The use of the Feulgen stain was not satisfactory for study with the electron microscope. The nucleus is clearly differentiated and reveals dispersed submicroscopic particles measuring about 250 Å. as well as hitherto undetermined structural organization of submicroscopic dimensions. Because of difficulties in 3-dimensional reconstruction and the necessity of additional work of an essentially technical nature, discussion of the genetical implications of these discoveries in vegetative and sporulating cells is currently premature. (Supported by the National Cancer Institute, The Commonwealth Fund, and the CLARA A. and WALLACE C. ABBOTT Memorial Fund of the University of Chicago.)

NANNEY, D. L., California Institute of Technology, Pasadena, Calif., and University of Illinois, Urbana, Ill.: *Serotype determination in Tetrahymena pyriformis, variety 1.*—Potentialities for the high temperature serotypes, 14H and R, described by MARGOLIN *et al.* (1959) for *T. pyriformis*, segregate in crosses as if controlled by allelic genes. However, the expression of these genes in heterozygotes involves a mechanism of mutual exclusion or "indeterminate dominance". Heterozygous cells removed from conjugation by only 10–15 cell generations usually manifest both serotypes, but to different degrees. By 100 cell generations 80–90% of the cells manifest only 14H or R. The patterns of intraclonal variation and the rates of fixation of pure types are essentially identical to those observed with mating type "stabilization" in this organism (ALLEN and NANNEY 1958) and require the same explanation. Some of the diploid and genetically equivalent subnuclei in heterozygous macronuclei are differentiated to express the R serotype; others are differentiated to express the 14H serotype. The diverse subnuclei are then passively assorted during cellular proliferation, and pure sublines are produced by "intranuclear epigenetic drift". Differentiations at the subnuclear level appear irreversible. No cytoplasmic effects on these nuclear differentiations have been discovered. Environmental effects have not yet been explored. (Supportd by a Senior Post-doctoral Fellowship from the National Science Foundation.)

NEWCOMER, EARL H., Botany Department, University of Connecticut, Storrs, Conn.: *A cytological demonstration of an X-ray induced chromosomal translocation in the domestic fowl.*—The cytological appearance and behavior of an X-ray induced reciprocal translocation between the first and second chromosome of the domestic fowl is described and its relevance to the further definition of linkage studies and chromosome mapping in the fowl is observed.

NICOLETTI, B., and D. L. LINDSLEY, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Reciprocal translocations between the X and the Y chromosome in Drosophila melanogaster.*—X/Y sperm from $X/sc^8 \cdot Y/Y \cdot B^S$ males were irradiated in order to induce reciprocal translocations between the X and the Y chromosome. X/X/Y daughters of the irradiated males were mated individually and their progeny scored for the presence of T(X;Y). The translocations were characterized by linkage between the irradiated X and Y. Approximately one percent of X/Y sperm irradiated with 4000r carried a newly induced T(X;Y)$^1$S.—The break point distribution along the X chromosome has been cytologically and genetically determined; breaks seem to occur preferentially in the proximal quarter of the X. The two arms of the Y chromosome seem to be involved with approximately equal frequency.—The majority of these translocations are male sterile or male lethal, although the male sterility may in some cases be covered by an extra normal Y chromosome. Anticipating this we designed the crosses so that the translocations were initially recovered in females.—Since these experiments produce X chromosomes broken at different points along their length, the duplications and deficiencies derived from them provide valuable tools for other studies.

ORIAS, E., (Introduced by David L. Nanney), University of Michigan, Ann Arbor, Mich.: *The mechanism of mating type determination in variety 8, Tetrahymena pyriformis.*—Variety 8 of *Tetrahymena pyriformis* shows the same pattern of mating type determination as *Paramecium bursaria* and as variety 2 of *T. pyriformis*. This pattern is characterized by (1) an almost perfect correlation in mating type between the four caryonides of a pair—caryonide is the clone whose members share, by common descent, one of the four macronuclei developed during conjugation—and (2) a high frequency of parental mating types among the progeny of a cross.—The underlying basis for this pattern has now been shown to be genetic for the case of variety 8. The three mating type specificities found in nature are controlled by three alleles of the "*mt*" locus. The pattern of dominance is such that a clone carrying at least one $mt^A$ allele exhibits mating type I; a clone carrying at least one $mt^B$, but no $mt^A$, allele exhibits mating type III, while the homozygote for the most recessive allele, $mt^C$, exhibits mating type II. Since the organism is diploid, six genotypes are possible with these three alleles, and they have all been synthesized.—

Slight deviations, both qualitative and quantitative, from expectations have been detected in several crosses, and are best explained as due to a high rate of "mutation" of $mt^A$ to $mt^C$. More spectacular deviations are found in crosses of the selfers—clones within which mating occurs—which arise in low frequency in many crosses. Under investigation is the possibility that these deviations result from the presence of unstable alleles of the *mt* locus in the germ line, which breed true vegetatively, but which are capable of stabilization at the time of meiosis. If true, this phenomenon may be related to the instability of mating type expression detected only in somatic nuclei of other Ciliates (e.g., variety 1 of *T. pyriformis*).

OSTER, I. I., and M. C. SUTCLIFFE, The Institute for Cancer Research, Philadelphia, Penna.: *The mutagenic effects of quinacrine mustard.*—Recently it has been reported by O. G. and M. J. FAHMY that certain amino acid analogues of nitrogen mustard exhibit specificity in regard to their effects on specific cell types and chromosome regions in Drosophila. To further investigate these effects genetic experiments have been undertaken with analogues of nitrogen mustard containing a quinoline (antimalarial type) moiety. These compounds have been chosen because they are strong alkylating agents, have a strong affinity for nucleic acid, have been shown to possess mitosis- and tumor-inhibiting properties by H. J. CREECH, and have specific pharmacological effects due to the carrier portion of the nitrogen mustard. They also possess the advantage of being capable of having considerable modifications made in the ring and side chain structures of the quinoline carrier of the nitrogen mustard with a view toward increasing the activity and/or selectivity of the mustard group. As a preliminary step it was necessary to show that these compounds are mutagenic when applied to Drosophila germ cells. Adult males (1–2 days old) were injected with .01M quinacrine mustard (2-methoxy-6-chloro-9-[4-bis($\beta$-chloroethyl)-amino-1-methybutylamino]acridine) and were mated to virgin females for three days. In the treated series the frequencies of lethals and translocations were 1.6% (8/511) and 0.4% (1/252) respectively, while untreated flies yielded 0.4% (6/1537) lethals and no translocations (0/1252). These results indicate that quinacrine mustard is mutagenic in Drosophila, being approximately as effective as the parent compound, nitrogen mustard. The relative efficacy with which this mustard compound and related derivatives produce different types of genetic damage will be discussed.

PIPKIN, SARAH BEDICHEK, Howard University, Washington, D.C.: *Sex balance in Drosophila melanogaster: aneuploidy of long regions of chromosome 3.*—Triploid aneuploid studies of long regions of chromosome 3 confirm the findings of short region studies previously reported in that no shift toward intersexuality is found in any hypertriploid female carrying in excess of 3X3A a long section of chromosome 3. Either the left end fragment or the right end fragment of two different translocations, T(3,4) 85C and T(3,4) 89E, broken at 85C and 89E, respectively, may be added to the 3X3A complement, producing hypertriploid females. In addition the left end fragment of TA(3,4) 12, broken at 73C, plus 3X3A results in a hypertriploid female. Hyperintersexes, with 2X3A plus either 85C L, 85C R, 89E R, or 12 L are usually of the extreme male type intersex, but the number of teeth in the sex combs averages fewer than in control intersexes. Long interior regions of chromosome 3 have been studied, but when the section exceeds 200 mm of BRIDGES' salivary map drawing, the overlap method breaks down owing to confusion of possible aneuploids with forms arising from nondisjunction of all parts of both translocations in the male parents heterozygous for two different translocations. In no instance was a sex shift found in hypertriploid females or in hyperintersexes for long interior regions of chromosome 3. Intersexuality in ordinary 2X3A triploid intersexes must therefore be dependent upon a dosage change in both the 2 and 3 chromosomes since present aneuploid studies of long and short regions of chromosome 3 alone or of chromosome 2 alone (PIPKIN 1947) do not show a sex shift in hypertriploid females. (This work was supported by Grant C-3453 awarded by The National Cancer Institute, U.S. Public Health Service, Bethesda 14, Md.)

RAVIN, A. W., University of Rochester, N.Y.: *The sites of mutations causing impaired synthesis of capsular polysaccharide in Pneumococcus.*—From encapsulated strains of Pneumococcus have been isolated a number of mutants of independent origin characterized by impaired synthesis of the specific capsular polysaccharide. These mutants fall into four distinct phenotypic classes: full capsule (N), reduced capsule (2), incomplete capsule (1), and no detectable capsule (R). Mutants of the former two classes may be distinguished from each other and from mutants of the latter two classes by the morphology of the colonies they produce. Mutants of the latter two classes are not distinguishable on the basis of colonial morphology, but can be separated on the basis of response to anti-R sera.—DNA-mediated transformations may be performed to determine the genotypic similarity of any two capsular mutants. While the DNA from cells of one mutant strain always fails to induce encapsulated transformants in cells of the same mutant clone, it may induce such transformants in cells of a different mutant clone. Depending on its origin (whether derived from an N-type or a 2-type cell), an R-type or 1-type mutant will produce characteristically either N-type transformants, or 2-type transformants, or both types of transformants, in response to the DNA of a different R-type or 1-type mutant.—Experiments have been designed to determine the relative frequency, at the cellular level, of transformation to a particular encapsulated type, using as an index the frequency of transformation to some other character (such as erythromycin- or streptomycin-resistance) or to some other encapsulated type. Results of such experiments reveal that most of the mutations resulting in impaired capsular synthesis occur at sites that are closely linked to each other in the bacterial genome. From transformations involving certain pairs of recipient and DNA-donor strains, it may be possible to map the order of the mutated sites in a postulated linear sequence.

ROMAN, H., and D. C. HAWTHORNE, University of Washington, Seattle, Wash.: *Reciprocal vs. nonreciprocal recombination in mitotically-dividing cells of yeast.*—We have a system in yeast which permits us to identify all of the products of an individual recombinational event occurring in mitotically dividing cells. We are looking for evidence of the reciprocality *vs.* the nonreciprocality of the recombinational event. Recombinations in regions between marked loci are principally reciprocal whereas allelic recombination (gene conversion) is principally nonreciprocal. Moreover, the response of each type of event to ultraviolet light is different: the frequency of allelic recombination increases exponentially with UV dosage whereas the response of recombination between loci appears to be linear. The evidence is in support of the hypothesis that the two events are due to different mechanisms.

RUDKIN, G. T., Institute for Cancer Research, Philadelphia 11, Penna.: *A comparison of the incorporation of tritiated thymidine and cytidine into giant chromosome puffs.*—Swollen regions (puffs) of the giant chromosomes in Dipteran larvae have been found by many workers to accumulate disproportionately large amounts of one or some of the three basic chromosomal constituents, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein. When, as was the case in a puff in section 60 of the second chromosome (2R) of *Drosophila melanogaster*, little or no quantitative changes are found to accompany puff formation, the possibility remains that rapid synthesis is counterbalanced by equally rapid loss of the product from the chromosome. For the present study, a tritium labelled nucleic acid precursor ($H^3$-thymidine or $H^3$-cytidine) was injected into late third instar Drosophila larvae and incorporation into the two major nucleic acid fractions of chromosomes was studied by autoradiography. Exposure to the isotope was for one to seven hours followed by fixation in 45 percent acetic acid. During the exposure period puparia were formed, puffs appeared in some chromosome regions and regressed in others, and still other regions remained puffed throughout the period. In all cases, puffs present in the chromosomes of prepupae were selectively labelled when tritiated cytidine was administered, indicating a relatively high turnover in the ribonucleic acid portion of the puffs. Very few chromosomes were labelled by $H^3$-thymidine although puffs did form, showing that synthesis of DNA is not necessary either for puff formation or maintenance. Comparison of grain counts

over puffed and non-puffed regions into which $H^3$-thymidine was incomporated confirmed the earlier conclusion that DNA synthesis is not disproportionately large in these puffs. On the contrary, the number of grains over the puff regions was lower than would be expected if incorporation were proportional to the relative ultraviolet absorbency of the puff region (puff to nonpuff, observed: 13.5:4.8; expected: 11.2:7.1 in thirteen chromosomes). The difference is not statistically significant, but is qualitatively supported by the paucity of grains observed over other puffs for which relative absorbency data are not yet available. The possibility suggested by these data that the synthesis of DNA in a chromosome may be affected by coincident synthesis of RNA is being investigated.

RUSSELL, LIANE BRAUCH and JEAN W. BANGHAM, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Variegated-type position effects in the mouse.*—Some interesting cases of mottling have occurred among the offspring of X-irradiated wild-type males mated, during their presterile period, to multiple recessive females. Testing of two of these mottleds indicates that they result from sex-linked translocations giving variegated-type position effects. Both mutants were semisterile females which, when mated to the appropriate recessive, produced more mottled progeny, all of which also were semisterile females.—One of the mutants was diffusely mottled with brown. The following explanations for offspring obtained by crossing mottled females to nonrelated brown males are consistent with the results. Mottled females are heterozygous for brown and a reciprocal translocation between chromosome VIII and the X-chromosome, the break in VIII being near the *b* locus, which carries the wild type allele. Males heterozygous for this translocation are black, rather than mottled; and completely sterile, rather than semisterile. Sterility is due to degeneration of spermatocytes in pachytene (OAKBERG). The segregants bearing the intact chromosomes are fertile brown males and females. Crossing over between the *b* locus and the breakpoint occurs with a frequency of about 15%. It results in semisterile brown females, sterile brown males, and fertile black animals of both sexes.—The other mottled mutant apparently involves an analogous situation, with the break presumably near the *c* locus.—These cases indicate that variegated-type position effects occur in mammals and that the X chromosome may be particularly effective in producing them.

ST. LAWRENCE, PATRICIA,[1] Yale University, New Haven, Connecticut and Stanford University, Stanford, California: *Gene conversion at the nic-2 locus of Neurospora crassa in crosses between strains with normal chromosomes and a strain carrying a translocation at the locus.*—In the niacin-requiring strain *4540T*, a segment of the chromosome of linkage group I is translocated to a subterminal position in the right arm of linkage group III. From crosses of *4540T* to stocks with normal chromosome complements, the parental types and a new class of progeny which carries a duplication of the translocated segment are recovered. Inviable spores are also produced. Segregation of the rearranged chromosomes and their normal homologues yields offspring showing 2:1 or other aberrant ratios for mutants in linkage groups I and III. From crosses between *4540* strains, however, 1:1 ratios of linked markers are obtained.—It has not been possible to separate the niacin requirement from the rearrangement and it appears likely that the nutritional deficiency is occasioned by a position effect. The failure to obtain reversions of *4540T* to prototrophy with either U.V. or X-ray treatment supports this view.—Rare niacin-independent progeny are recovered from crosses of *4540T* to non-rearranged alleles at the locus. Evidence that these exceptional offspring result from gene conversion is derived from analyses of the segregation of linked markers. Since the *4540T* stock has a rearrangement at the *nic-2 locus*, there must be an interruption of pairing at or near this region in these crosses. It is therefore suggested that intimate pairing may not be a *sine qua non* for gene conversion. (Supported in part by a grant to D. M. BONNER from the American Cancer Society on recommendation of the Committee on Growth and in part by research grant E1462 to D. D. PERKINS from the United States Public Health Service.)

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SAND, SEAWARD A., Connecticut Agricultural Experiment Station, New Haven, Conn.: *Effects on progeny segregation following chronic gamma irradiation of the mutable V and stable R loci in a clone of Nicotiana.*—Clone S5230–5, selected from cultures of interspecific hybrid origin, was employed. This clone is heterozygous for the mutable *V* gene ($v_{s3}/v_s$) and for the stable *R* gene (*R/r*), which affect flower color. Growing plants were subjected to different levels of chronic gamma irradiation at Brookhaven National Laboratory. The somatic effect on a mutable gene system was compared with a stable gene system in the same tissue by counting speckled sectors (changes from $v_{s3}$ to $v_s$ phenotype) and purple sectors (changes from *R* to *r* phenotype) on irradiated plants. Similarly, germinal effects on the same gene systems were studied by counting speckled and purple individuals in progeny from the irradiated plants.—The somatic response curve for the mutable gene is about ten times greater in slope than the purple sector response in the treatment range below 12 roentgens per day (SAND, SMITH and SPARROW 1954). However, the germinal response curves are similar for the mutable and stable genes. Chronic gamma irradiation of this heterozygous clone increases the frequency of recessive individuals in progeny grown under nonirradiation conditions. The major increment is confined to the low dosage range. Above about ten roentgens per day these data show no further significant effect on progeny segregation beyond that already produced at this lower dosage rate.—The somatic and progeny results suggest (1) a mutational component of effect, and (2) a buffering system against mutation, sensitive to damage by low dosage rates of irradiation. The similar progeny segregation responses indicate equal potential mutability of $v_{s3}$ and *R* under conditions that remove secondary stabilizing influences.

SCHEINBERG, S. L., and R. P. RECKEL, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland: *Induced somatic mutations affecting erythrocyte antigens.*—Isotope dilution experiments with the bloods of the pigeon, *C. livia*, using *Phaseolus lunatus* extracts as the agglutinin, confirm the existence of the nonagglutinable cells observed in man but at a higher frequency, i.e., (.026–.1). The agglutinin specific for pigeon cells is believed to be anti-$A_2$ since pigeon cells do not react with anti-$A_1$ (*Dolichos biflorus*) but do react with eluates from human $A_2$ cells following absorption of *P. lunatus* extracts with $A_2$ blood.—The frequency of nonagglutinable cells was obtained on the bloods of four pigeons prior to and following a total body X-ray exposure of 700–1000r. Three of the pigeons examined at about 40 days postirradiation showed increases in the frequency of nonagglutinable cells from 35–75% over the initial frequency. At about four months the bloods of three pigeons were re-examined and the increase over the preirradiation frequency ranged from 50–82%. The fourth pigeon was subject to treatments which differ from the others and data concerning that pigeon will be discussed.—Blood was obtained from a patient* with polycythemia vera treated with 2 mc of $P^{32}$. Seven days following treatment the frequency of A negative cells in this patient was .0021, after 48 days this had risen to .016 and was .018, 91 days later.—It is concluded that irradiation has resulted in damage to the bone marrow stem cells and provides additional evidence for the mutational origin of the antigen lacking cells.—* The authors are indebted to DR. SNYDER and his patient for samples of blood.

SCHNELLER, MYRTLE V., T. M. SONNEBORN, and JO ANNE MUELLER, Indiana University, Bloomington, Ind.: *The genetic control of kappa-like particles in Paramecium aurelia.*—Kappa in killer *P. aurelia* of syngen 4 is known to depend principally upon the presence of gene *K*; homozygotes for *k* soon lose their kappa irreversibly. A killer stock (239) of syngen 4 has now been found which is homozygous for gene *k'*. This killer contains cytoplasmic particles which are two to four times as large as those in other killers of syngen 4, and which lack the refractile body, characteristic of killing particles in other stocks. The killing action of stock 239 is very different from that of other killers of syngen 4, but is identical with that of SCHNELLER's (1958, Proc. Indiana Acad. Sci. 67: 302) stock 299 of syngen 8. The latter killer (stock 299) was crossed to killer stock 214 (syngen 8), which kills in a way markedly different from all the preceding killers. The breeding analysis shows that the genome which supports the cytoplasmic particles of

stock 299 will not support those of stock 214 and vice versa. Stock 214 contains one principal gene (*l*) which, when introduced into stock 299, results in loss of its kappa-like particles; preliminary results indicate that stock 299 has more than one gene which has this effect on the particles of stock 214. There is no earlier information available on the genetic control of the particles in ordinary killers of this syngen (8); but the $\mu$ particles of mate-killers are known to require gene *M*. In sum, it appears that there is a complex set of relations between the genome of the paramecia and the kinds of cytoplasmic particles: each kind of particle requires the presence of certain genes in the paramecium nucleus, different kinds of particles requiring different genes. (Supported by grants to T. M. SONNEBORN from American Cancer Society, Atomic Energy Commission and Rockefeller Foundation.)

SCHURIN, M. F., (Introduced by CYRUS LEVINTHAL), Massachusetts Institute of Technology, Cambridge, Mass.: *Localized cytochemical and submicroscopic differentiations in Drosophila virilis salivary gland chromosomes.*—Cytochemical determinations on *D. virilis* salivary gland nuclei have localized the four major chromosomal constitutents and suggested correlations with electron microscope observations have been proposed. The histone distribution (method of ALFERT and GESCHWIND 1953) closely parallels that of the DNA, but the nonhistone protein (DEITCH 1953) has a completely independent pattern. Quantitative comparisons of the DNA and histones have been done by recording densitometry on subsequent staining of the same nucleus. The DNA and chromosomal RNA have been visualized simultaneously, utilizing the metachromasia of Azure B (FLAX and HIMES 1952). The RNA occurs discretely in specific bands and puffs. Analysis of a specific puff (end of chromosome II) indicates that puff formation is accompanied by a constancy of DNA and histone, but a marked increase in RNA and nonhistone protein. Similarly, the nucleolus contains no DNA or histone but only RNA and nonhistone protein. Under the electron microscope the chromosomes normally appear fibrous, but frequently localized areas of large granules (diameters 900–3000 Å) are seen, both in discrete bands and large masses within the chromosomes. These large granules are composed of agglomerations of small granules (100–200 Å in diameter) intimately connected with the individual fibers. It is suggested that these granules are the morphological manifestation of the RNA bands and puffs and thus are the chromosomal counterpart of cytoplasmic ribosomes. The similarity in cytochemical composition and fine structure of the RNA bands, puffs, and the nucleolus, indicates that these are different degrees of the same genetic synthetic process involving the accumulation or production of RNA—nonhistone protein granules.

SEARS, E. R., U. S. Department of Agriculture and University of Missouri, Columbia, Mo.: *Neatby's virescent, a chlorophyll aberration in common wheat.*—A mutable recessive gene that causes a virescent condition in *Triticum aestivum* was reported by NEATBY in 1933. Several virescent (*vv*) plants showed a green sector, and among the offspring of *vv* were one green and one albino plant.—Aneuploid analysis shows that *v* is an active allele that has little or no effect in one dose, causes virescence in two doses, and results in albinism in three doses. Growth is retarded, also, especially at the three-dose level. The apparent mutability of the gene is evidently the result of mere nondisjunction of chromosome III, which carries *v*.—When the related (homoeologous) chromosome XVI is made monosomic, *vv* is lethal to the embryo; and when XVI is tetrasomic (two extra doses), *vv* plants are green. The other homoeologue, XII, has an effect when monosomic similar to mono-III, except that some mono-XII *vv* individuals are weakly viable to maturity. There are thus genes on chromosomes XII and XVI which compete with *v*. *V*, the normal allele of *v*, apparently is similar in this respect to the genes on XII and XVI, for mono-XVI *v*–, but not *Vv*, is an embryonic lethal. The possibility is being tested that chromosome XII and XVI carry genes $V_2$ and $V_3$, respectively, which are essentially duplicates of $V_1$ on chromosome III. The three genes have the same function but differ somewhat in strength. They are involved in chlorophyll formation.

SHULT, E. E., C. C. LINDEGREN and SHARON DESBOROUGH, Southern Illinois University, Carbondale, Ill.: *The application, to tetrad-analysis-data from Saccharomyces, of principles for establishing the order of genetic factors.*—Empirical criteria utilized in ordering genetic loci define, rather than prove, linear order. The degree of certainty of linear order has its source in the mutual confirmation of a series of independent applications of the criteria. That the single application of an ordering principle to one set of 3-point data does not comprise a demonstration of linear order is proved by either of the following: (1) inconsistencies in gene order when the principle is applied to a linkage group containing four or more loci, and (2) the existence of nonlinear gene arrangements.—Three ordering criteria are defined and applied to five genes of chromosome I in Saccharomyces. On the basis of ten sets of 3-point tetrad data, the linear order *UR-CH-HI-IS-AN* is defined by all three ordering principles. Of the 30 independent tests: three tests define the order of the five loci; 21 tests confirm this order; two tests contradict it; and four tests are inconclusive. It is shown that the exceptions imply that interregional chromatid interference is stronger than that which operates intraregionally. This is supported by a direct analysis of double recombinations, which exhibit significant chromatid interference between compound regions, but subsignificant interference between adjacent simple regions.—The gene, *TH*, is shown to be a member of linkage group I, but departs significantly from a linear arrangement. (The *TH*-arm branches from chromosome I near the centromere, between *UR* and *CH*, and the "branch" is inferred to result from preferential segregation of centromeres to produce "quasi-" or "pseudo-linkage.")

SIEGEL, A., Botany Department, University of California, Los Angeles 24, Calif.: *The induction of mutations of tobacco mosaic virus with nitrous acid.*—Recently GIERER and MUNDRY (1958) have demonstrated that nitrous acid treatment of tobacco mosaic virus or of its free nucleic acid will induce the appearance of mutations. Their results have been confirmed both quantitatively and qualitatively in the present study. In addition the following observations have been made: (a) Local necrotic lesion size on *Nicotiana glutinosa* is a character of the virus that is highly mutable. For every three events that lead to the inactivation of a virus particle, approximately one event occurs which leads to a small lesion producing particle. This class of mutation appears to be unidirectional. No isolates which produce lesions larger than the control have been observed. (b) Other types of observed mutations are an alteration of symptom in tobacco plants and a change from systemic invasion to necrotic lesion production in *N. sylvestris*. The total frequency of observed mutations suggests at least one mutating event for every two killing events. (c) No mutants have been observed with altered protein, as judged by serological specificity and isoelectric point, even after treatment which yields 94% frequency of "biological" mutants. (d) Strains of the virus differ in their susceptibility to mutation with nitrous acid treatment. Strains U1 and U4, which have the same sensitivity to inactivation with nitrous acid, differ markedly in the frequency with which mutants can be detected among the survivors of equivalent treatment.

SIEGEL, R. W., and L. LARISON, University of California, Los Angeles, Calif.: *Mating type inheritance and determination in Paramecium bursaria.*—The present reanalysis of mating type inheritance and determination in *P. bursaria* was evoked by the alternative hypotheses of JENNINGS and SONNEBORN.—Data from an extensive breeding analysis carried out by JENNINGS some 15 to 20 years ago led him to suggest that the various mating types present among an interfertile group of clones (="syngen" or "variety") are controlled directly by genotypic differences. However, neither JENNINGS nor others were able to devise workable genetic formulae to account for his results. More recently, SONNEBORN has advanced the idea that mating type is directly determined by the relative concentrations of several "cytoplasmic differentiators," the expressed mating type corresponding to that differentiator initially present in the highest concentration; the hierarchy of cytoplasmic differentiators is presumed to be controlled by unspecified genotypic and environmental factors.—Four highly interfertile strains, representing the

four mating types of syngen 1, were recently isolated from Malibu Lake, California. Crosses among these strains reveal the following: First, conjugants do not regularly exchange massive amounts of cytoplasm as required by SONNEBORN (but not JENNINGS) to account for the observed identity among the progeny from a given pair. Second, there is no marked influence of temperature on mating type determination. Finally, the results of all crosses analyzed to date lead us to postulate the direct control of mating types by two loci; clones with dominant alleles at both loci (*A–B–*) are type A; homozygous recessives (*aabb*) are type C; types B and D are respectively *aaB–* and *A–bb*. This hypothesis (actually considered by JENNINGS in 1942) satisfactorily accounts for the results of JENNINGS' interstock crosses; however, the determination of mating type for certain other clones in which instability during vegetative reproduction ("self-differentiation") was found, remains enigmatic. (Supported by a grant from the National Science Foundation and by Cancer Research Funds of the University of California.)

SISKEN, JESSE E., City of Hope Medical Center, Duarte, California: *DNA synthesis and the mitotic cycle as studied by the combination of autoradiography and microcinematography.*—A technique has been developed which combines time-lapse microcinematography with autoradiography of the same cells grown *in vitro*. Cells grown on the glass coverslips of Rose chambers were photographed for periods up to 36 hours to record their mitotic history. Ten minutes prior to the end of photography, the medium was exchanged for one containing tritiated thymidine. Immediately following photography the cells were fixed *in situ* by exchanging Carnoy's fixative for the medium. The coverslip bearing the cells was taken through the Feulgen staining procedure and the autoradiograph prepared. Individual cells could be found both in the autoradiograph and in the motion picture, so that the timing of incorporation into DNA in relation to the mitotic cycle could be determined. Time between the beginning of anaphase and the beginning of incorporation into DNA varied somewhat in different subcultures of NAKANISHI's kitten lung cells. There was little variation within a single subculture, however. In order to give more meaning to these experiments, long term motion pictures (60–77 hours) have been analyzed to determine directly the length of the cell cycle by following individual cells from one division to the next. Data will be presented showing variation in the time of the cell cycle which is dependent upon culture conditions. In addition, observations on the range of variation between two daughter cells of the same division will be discussed.

SLATIS, H. M., Argonne National Laboratory, Lemont, Ill.: *Anomalous development of the tracheae in Drosophila melanogaster larvae.*—A respiratory mutant of Drosophila larvae in which portions of the main tracheal trunk are absent has been given the name tracheae broken (*tb*). It appears to be a spontaneous mutant of poor penetrance. In a large proportion of the cases, the absence is of the first abdominal segment on one side, beginning just posterior to the anterior crossbranch. The main tracheal trunk may be affected in any other segment, including the absence of a spiracle. Loss of the tracheae in two places may occur unilaterally or bilaterally, apparently with random frequency. In the absence of a portion of the main tracheal trunk there is a change in the secondary tracheal system which increases the amount of anastomosing between the segments surrounding the affected region. The resulting tracheae appear to comprise an efficient respiratory system and the viability of *tb* larva originally observed in first instar does not seem to be impaired. The condition is temperature sensitive, varying from 5% of larvae developing at 25° to 15% of those developing at 16°. (Work performed under auspices of U. S. Atomic Energy Commission.)

SOLBERG, BJØRN, and ELLSWORTH C. DOUGHERTY, Kaiser Foundation Research Institute, Richmond, Calif.: *Male-producing (normal) lines and a maleless (aberrant) line in the rotifer Brachionus variabilis.*—Since late 1957, a strain of *B. variabilis*, isolated from a sewage oxidation pond, has been cultured continuously in our laboratory (DOUGHERTY and SOLBERG, Anat. Record, abstract in press). In April, 1958, a dixenic culture (rotifers + *Chlorella pyrenoidosa* + Gm–negative, coliform bacteria of an unidentified species, all in Seitz-filtered pond water)

was established. Thereafter for successive generations we selected and individually isolated, each generation, progeny of the earliest born rotifer to give amictic (parthenogenetically developing, ♀-producing) eggs. In the main (M) line all progeny of such first born rotifers were not uniformly saved. Sublines (A and B) were initiated with two females hatched from sibling dormant eggs laid by a sixth generation mictic M ♀ mated with one (? or more) of her nephews. The A line resembled the parent M line in yielding both amictic and mictic eggs; of 280 A rotifers selected in 18 unbroken generations, 259 survived to produce eggs—44% amictic, 56% mictic. The B line, however, produced *no* mictic eggs in 24 generations (161 rotifers), interrupted once for one generation; nor have such eggs been seen to date in any mass B culture. The A line averagely yielded 15.6 offspring/generation; the B line only 6.7 (43% of A level). Since the reduction in B fertility corresponds to the percentage of mictic females in the A line, perhaps the B malelessness is linked to a genetic condition harmless to cells of the germ line fated to become amictic eggs, but lethal to those fated, in normal lines, to become mictic eggs. Cytological studies are needed to check this. (Supported, in part, by Grant G-6018 of the National Science Foundation.)

SPIESS, ELIOT B., Department of Biological Sciences, University of Pittsburgh, Pittsburgh 13, Pa.: *Lack of heterosis in experimental populations of Drosophila persimilis from Timberline in the Sierra Nevada.*—Descendants of flies from a 10,000 ft. elevation locality collected in 1954 by DOBZHANSKY were introduced into eight population cages at 16° C. Each cage was initiated with two of the three commonest gene arrangements (Whitney, Klamath, and Mendocino, which occurred respectively at about 90%, 4%, and 5% at Timberline). Three populations containing WT *vs.* KL show constant drop in frequency of KL to near elimination after ten generations. WT/WT must therefore be superior to WT/KL. Three populations of WT *vs.* MD show no changes for the initial six generations. Thereafter MD changes frequency but in different ways in the two long term populations. Apparently all zygotes of WT and MD are adaptively equal but differences develop after the sixth generation. Two populations containing KL *vs.* MD show no easily interpreted changes: there is no consistent equilibrium attained after ten generations. These results contrast with population results with flies from White Wolf (Evolution 11: 84–93) in which heterokaryotype superiority and adaptive value dependency upon frequency was displayed. Heterokaryotype superiority (WT/KL or WT/MD) at 16° C has never been lacking in populations of *D. persimilis* from lower elevations studied formerly. Evidently adaptive complexes in Timberline flies are so different that laboratory conditions do not evoke a response of balanced adaptive changes expected on the basis of experiments with lower elevation flies. (Work supported under Contract AT (30–1–1775, U. S. Atomic Energy Commission.)

SPOFFORD, JANICE B., University of Chicago, Chicago, Ill.: *Residual effects of parental X chromosomes on white-variegation in offspring.*—Extra Y heterochromatin in *Drosophila melanogaster* has long been known to restore partially the normal expression of a euchromatic locus subject to position-effect variegation when in the vicinity of broken heterochromatin. Some portions of the Y heterochromatin in mother's genome but not in offspring's genome have been shown to increase normal expression of the $w^+$ locus in the offspring as well, presumably through effects on the oöplasm, at least for an insertion carrying $w^+$ into the proximal heterochromatin of 3L (Dp($w^m$) 264.58a).—With X·Y (attached-XY) fathers and X·X (attached-X) mothers, offspring receiving the Dp from the father are much more heavily pigmented and/or are much more likely to have any eye pigment than those receiving a maternal Dp. The same holds for offspring of X/Y and X·X mothers. However, there is no effect of parental Dp-source on daughters of X/Y fathers and X/X mothers; the likelihood of sons having pigment is somewhat greater if the Dp was maternal. Furthermore, no X·X/O; Dp/+ female has been found to display a trace of eye pigment, while X/X; Dp/+ females often do; there is 100% penetrance in the latter when their mothers were homozygous for Dp. Consequently, the $y\ w$ attached-X chromosome appears to be responsible for some inhibition of the action of the $w^+$ gene in the rearrangement, an inhibition which persists in the egg after removal of the chromosome itself

by the maturation division. (This research was supported by Contract AT(11–1)-431 with the Atomic Energy Commission.)

STADLER, D. R., University of Washington, Seattle, Wash.: *The relationship of gene conversion to crossing over in Neurospora.*—The production of infrequent wild type progeny in crosses between two allelic or closely linked mutants of similar phenotype has been termed gene conversion. Two general types of hypothesis have been advanced regarding the relationship of this process to crossing over: (1) the two are separate events; (2) they are both results of the same recombination event. The single event hypothesis proposes that gene conversion reveals what happens at the site of the event which may result in crossing over with regard to markers on both sides. According to the single event hypothesis, one might predict that a recombination event detected by either gene conversion or crossing over should have the effects associated with recombination events of any kind. One effect of crossing over is interference with crossing over in neighboring regions. An experiment has been performed to study the relationship of gene conversion to crossing over in a separate but nearby chromosome region. The single event hypothesis might predict interference in all the converts, even those with no recombination for the markers embracing the conversion locus. The separate events hypothesis would predict no interference in this group.—Cysteine-independent converts were selected from the cross *lys*+ *cys-t ylo*+ *ad* × *lys cys-c ylo ad*+. Crossing over frequencies were: *lys* 4.1 *cys* 19.2 *ylo* 10.7 *ad*. Of 84 converts with nonparental combinations of adjacent markers (*lys*+ *cys*+ *ylo* and *lys cys*+ *ylo*+), one had *ylo-ad* recombination. Of 96 converts with parental combinations of adjacent markers (*lys*+ *cys*+ *ylo*+ and *lys cys*+ *ylo*), 16 had recombination in the *ylo-ad* interval. The results suggest that gene conversion and crossing over are separate events.

STADLER, JANICE, Iowa State College, Ames, Iowa: *Cataract in mice as affected by radiant energy.*—For some years a cataract stock of mice, blind at birth, has been maintained in our laboratory. Descriptions of this condition have been given by F. L. P. KOCH and JOHN W. GOWEN 1939. Crosses have been made reciprocally between this stock and our inbred BALB/Gw stock. The resulting reciprocal $F_1$ hybrids have been exposed to X-rays from 98 pkv 25 ma source along with mice of the inbred line at 46 days of age. All the $F_1$ animals have normal vision but may be regarded as carrying a genetic predisposition to cataract. The inbred line to an age of 250 days was not known to be prone to cataracts. Mice of the three genotypes were exposed to X-ray dosages of 0, 160, 320 and 480r, in equal numbers. The results show like predisposition to cataracts for the reciprocal $F_1$ animals over their lifetimes. The inbred strain on the other hand, develop nearly twice as many cataracts as did the $F_1$'s. There is no evidence for a known hereditary predisposition to cataracts influencing the phenotypic appearance of this condition when such animals are irradiated. Increases in the radiation dosages increased the frequency of cataracts in nearly the ratio of the second power of the dose. Cataract appearance was age dependent. No cataracts appeared before the animals were 300 days of age. There is a dose-age interaction, such that at higher dosage, cataracts appear earlier. The sexes were equal in cataract frequency. (This work has received assistance from Contract Number AT(11–1) 107 from the Atomic Energy Commission.)

STALKER, H. D., Washington University, St. Louis, Mo.: *Nonrandom associations of inversions in chromosomes of wild Drosophila paramelanica.*—*D. paramelanica* is structurally polymorphic in the X and Chromosome 2. In the X the commonest chromosome types are ++·++ (Standard) and chromosomes carrying the short X-left inversions A and B associated with the X-right inversions C and B, thus: AB·CB, (the two B inversions are not homologous). Such AB·CB chromosomes carry a "sex-ratio gene" of the type known in the *D. obscura* group. In heterozygous ++·++/AB·CB ♀ ♀ which are also structurally heterozygous for Chromosome 2, crossing over between any pair of adjacent inversions occurs with a frequency of roughly 0.1%. Thus, the various X inversions might be expected to show random association, with the frequency of each association predictable from the inversion frequencies in the given population. Samples

from ten localities in Mo., Ia, Minn., Mich., N. Y and Vt. showed that the frequency of the ++·++ and AB·CB chromosomes was above expectation (322 obs., 151 exp.) while all other combinations were present in frequencies below expectation (20 obs., 191 exp.). The consistent pattern of disagreement with expectation in all populations and the widespread occurrence of the various X chromosome types indicates that neither drift nor the retention of ancestral associations can explain the results. The conclusion favoring the selective elimination of all associations other than ++·++ and AB·CB is reinforced by the finding that associations which require two or more (presumably sequential) crossovers for their production are more strikingly deficient in populations than those which might be produced by a single crossover between ++·++ and AB·CB chromosomes. (Studies aided by a grant from the National Science Foundation.)

STEFFENSEN, DALE, LEO E. LACHANCE and NANCY A. STALEY, Brookhaven National Laboratory,[1] Upton, N.Y.: *Retention of calcium*$^{45}$ *in Habrobracon sperm.*—Recently calcium has been assigned a role in the structure of chromosomes (GENETICS 42: 239, 1957). Most of the evidence has come from cytological studies with plants grown on suboptimal calcium cultures or from work with chelating agents. The present study was proposed to examine the incorporation of calcium into nuclei of sperm using autoradiography. The parasitic wasp, *Habrobracon juglandis* (Ashmead), was chosen as favorable material because of the known localization of sperm nuclei in fertilized eggs. An additional advantage is that fusion of male and female nuclei occurs and embryonic development proceeds about an hour after eggs are laid.—Prestung Ephestia caterpillars were injected orally with solutions of $Ca^{45}$ ranging from 0.40 mc/ml to 0.097 mc/ml. These injected hosts were then placed in Stender dishes with virgin wasps. The larvae that hatched fed on labeled body fluid of the host. The high concentrations were lethal to the developing larvae. Eclosing males from lower concentrations were counted in a gas-flow counter and registered from 1000 to 7000 counts per minute per male. Radioactive males were then mated to virgin unlabeled females. The eggs by these females were collected at timed intervals, placed on slides, fixed in 70 percent neutral alcohol and prepared for autoradiography. After 2 to 4 months of exposure the resulting autoradiographs revealed that sperm nuclei retained the $Ca^{45}$ in nonradioactive cytoplasm, both before and approaching syngamy. The fate of the isotope during the cleavage divisions and DNA synthesis is still not known but is under study.—The sperm were birefringent as observed in polarized light indicating a parallel orientation of chromosomes similar to sperm of other invertebrates and vertebrates. These studies and unpublished experiments with Lilium pollen tubes would indicate that calcium is bound in the chromosomes' structure.

STEINITZ-SEARS, LOTTI M., University of Missouri, Columbia, Mo.: *Derivatives of a dicentric chromosome in wheat.*—From a spontaneous dicentric chromosome, which was present from root tip to meiosis, where it participated in the formation of a translocation trivalent, the following types of derivatives were obtained in the next generation: (1) A functionally monocentric chromosome in which the intercentromeric segment had presumably become so short as to be virtually absent. One plant had a pair of these and was thus nullisomic for arms of two different chromosomes of the standard line. (2) Two monocentric chromosomes, both usually recognizably deficient for part of one arm. These originate through breakage and healing of the dicentric. (3) A tricentric chromosome, found only in a small group of microsporocytes from a plant possessing the dicentric. This tricentric paired with two normal chromosomes, and with alternate orientation of the centromeres assumed a characteristic W shape. (Work supported by the National Science Foundation.)

STONE, W. H., S. ABRAHAMSON and JANIS BECKSTROM, University of Wisconsin, Madison, Wis.: *Immunogenetic studies of the J substance of cattle saliva.*—The J substance of cattle saliva can

[1] Under the auspices of the U. S. Atomic Energy Commission.

be detected by its ability to inhibit the reaction between antibodies for J and known J-positive erythrocytes. Inhibition tests of the serum and saliva of 162 adult cattle showed a complete concordance between the presence and absence of the soluble J substance; i.e., all of the 85 animals with J in their sera also had it in their salivas and the 77 animals lacking J in their sera also lacked it in their salivas. Further, the data indicated that the degree of J activity in the serum and in the saliva was correlated.—Of 31 matings between positive parents, there were 22 positive and 9 negative offspring. From 81 matings between positive and negative parents a distribution of 45 positive and 36 negative offspring was obtained. There were no positive offspring from 28 matings of negative parents. These preliminary data suggest that the presence or absence of the J substance of the saliva is controlled by a Mendelian dominant gene. As a consequence, an individual should not possess J substance in its saliva unless one or both parents possess it. Further studies on the quantitative aspects of this system are in progress.

STRAUSS, B., and S. OKUBO, Osaka University, Osaka, Japan: *The effect of metabolic processes on mutation induced by alkylating agents.*—The reversion to the prototrophic condition of a tryptophan requiring strain of *Escherichia coli* B/r has been studied after treatment with either ultraviolet (UV) light or with one of the alkylating agents diethyl sulfate or epichlorohydrin. The number of revertants obtained after treatment with the chemical mutagens is greatly increased by incubation for one hour at 37° in a medium supplemented with broth and tryptophan as is the case with UV induced mutagenesis. The quantitative effect of plating on medium supplemented with different amounts of broth differs depending on the mutagen. Incubation of UV treated cells in medium containing the analog tryptazan results in a large decrease in the yield of reversions when the cultures are subsequently plated on broth supplemented plates. Incubation of ethyl sulfate or epichlorohydrin treated cells in tryptazan containing medium results in a comparative decrease in the number of revertants obtained when plating is done on a minimal medium but this inhibitor causes *no* decrease in the number of revertants when plating is done on broth supplemented plates following the incubation.—We therefore conclude that the "premutated state" induced by the alkylating agents does not require immediate protein synthesis for fixation into the genome. The protein synthesis requirement for the fixation of mutations following UV first demonstrated by WITKIN and by DOUDNEY and HAAS may be an effect specific to the type of mutagen used rather than to the general process of mutation.

SUEOKA, NOBORU (Introduced by R. P. LEVINE), Harvard University, Cambridge, Mass.: *Replication of deoxyribonucleic acid in Chlamydomonas.*—Analysis of DNA replication in mitosis and meiosis of the unicellular green alga, *Chlamydomonas reinhardi*, is the main purpose of this investigation. The techniques used are the density gradient centrifugation and incorporation of a heavy isotope of nitrogen ($N^{15}$). At this writing the mitotic replication of DNA has been analyzed. The vegetative cells were grown in $N^{15}$ medium and transferred to $N^{14}$ medium. Samples of cells were drawn at various times after the transfer, lysed, and centrifuged. The results show that the three kinds of DNA molecules, fully labeled, half labeled, and nonlabeled, are clearly separated as narrow bands. The sequence of the appearance of these three is apparently from the fully labeled to the nonlabeled through the intermediate form, the half labeled. This indicates that in Chlamydomonas, a eucaryote, the mode of mitotic replication of DNA is identical with that of *E. coli* as analyzed by MESELSON and STAHL, and is best interpreted by the bipartite structure of DNA by WATSON and CRICK. The breakage and reunion between old and new subunits is infrequent, if at all. The conclusion is in complete accordance with the autoradiographic analysis of mitotic duplication of plant chromosomes by TAYLOR. The meiotic replication of DNA in Chlamydomonas is being analyzed. (This work is supported by grants to R. P. LEVINE and J. D. WATSON from the National Science Foundation and the United States Public Health Service.)

SZYBALSKI, W., and B. DJORDJEVIC, Institute of Microbiology, Rutgers, the State University, New Brunswick, N.J.: *Radiation sensitivity of chemically modified human cells.*—Earlier studies

(Federation Proc. 18: 336, 1959) concerning the effect of ultraviolet (UV) irradiation on the mutation rate from 8-azaguanine(AG)-sensitivity to resistance in cloned cell cultures derived from human bone marrow (D-98) indicated that sensitivity to the lethal effect of UV depends on several factors. The survival of irradiated cells was measured as the proportion of colony-forming cells. The AG-resistant mutants pre-existing in the AG-sensitive population (never exposed to AG prior to UV irradiation) exhibit a "single-hit" UV-survival curve, and thus differ from the established AG-resistant lines (exposed to AG) and from the wild type cells, both of which are characterized by "multihit" survival. Furthermore, cells grown for 2 to 4 days in the presence of inhibitory concentrations of AG or 5-fluorodeoxyuridine, prior to UV irradiation, become more radiation-sensitive.—Since it is known that 5-bromodeoxyuridine (BUDR) can replace thymidine in DNA, its radio-sensitizing effect on D-98 cells was evaluated. It was found that cells grown for 2 to 4 generations in the presence of 2–50 μg/ml of BUDR retain their viability, as measured by colony-forming capacity. In such cells, however, the resulting partial substitution of thymidine by BUDR dramatically increases the sensitivity to UV, and also to X-rays. The survival curves acquire a "single-hit" character and their slopes become steeper by a factor as high as eight. BUDR-effected radiation sensitization was also observed for phage φX-174 (presumably containing one molecule of single-stranded DNA), bacteria, and mice, but phage T2 did not exhibit this type of response. (Supported by Grant No. CY-3492 from the U.S. Public Health Service).

TAKATS, STEPHEN T. (Introduced by JAMES L. BREWBAKER), Biology Department, Brookhaven National Laboratory, Upton, N. Y.: *Cytoplasmic incorporation of thymidine during meiosis and premeiosis in Lilium longiflorum.*—Tritiated thymidine has been used to label the chromosomes in sporogenous cells both during the DNA synthesis before meiotic prophase and during earlier premeiotic syntheses when the cells are dividing mitotically. It has also been incorporated into anthers at leptotene and zygotene of meiosis. At all these stages autoradiographs show label in the cytoplasm. If tritiated thymidine is incorporated during the meiotic synthesis, cytoplasmic label persists at least until diakinesis.—Tentatively, this label has been determined to be in DNA, and to a small extent in protein. It is not removed with 10% perchloric acid at 20°C for 4 hours, nor with *N*HCl at 60°C for 10 minutes in the Feulgen procedure. It is largely but not completely removed by hot 5% trichloroacetic acid.—A correlation appears between the extent of cytoplasmic labelling and the occurrence of nuclear labelling in the tissue. When no nuclei synthesize nuclear DNA, as during leptotene and zygotene, the cytoplasmic label is at a minimum. It is also very low immediately preceding the meiotic synthesis, when the sporogenous cells stop dividing mitotically. Earlier than this, all cells show considerable cytoplasmic label, although the mitotic index and corresponding percentage of labelled nuclei is *ca.* 3%. These results are considered in relation to biochemical observations on mammalian liver which show that the phosphorylation and incorporation of thymidine into DNA proceeds much more rapidly with a cytoplasmic enzyme fraction isolated from regenerating liver than from normal liver. (Work supported by a Postdoctoral Fellowship from the National Cancer Institute, U. S. Public Health Service, and carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

TAUB, STEPHAN R., Department of Zoology, Indiana University, Bloomington, Ind.: *The genetics of mating type determination in syngen 7, Paramecium aurelia.*—The group B system of mating type determination in *Paramecium aurelia* is characterized by what appears to be cytoplasmic inheritance of mating type. However, SONNEBORN (1954, Caryologia, suppl. 1:307) showed (1) that the macronucleus controls mating type, (2) that macronuclear mating type differentiation is directed by cytoplasm transmitted from the previous sexual generation, and (3) that the cytoplasm is determined as to its effect on macronuclear differentiation by the macronucleus of one sexual generation in such a way as to maintain the same mating type in the next sexual generation.—TAUB (1958, J. Protozool. 5, suppl. :18) reported (1) that syngen 7 belongs to group B, (2) that certain stocks of syngen 7 contain a recessive gene, $mt^{XIII}$, which restricts

homozygotes to mating type XIII, and (3) that, unexpectedly, these homozygotes have a type XIV-determining cytoplasm, since, when the dominant allele, $mt^{XIII,\ XIV}$, which permits the expression of either mating type XIII or XIV, is introduced, the resulting heterozygote becomes mating type XIV.—Crossing this heterozygote to a XIII of genotype $mt^{XIII,\ XIV}/mt^{XIII,\ XIV}$ makes it possible to obtain the genotype $mt^{XIII}/mt^{XIII,\ XIV}$ in an animal of mating type XIII. Such mating type XIII heterozygotes prove to have a type XIV-determining cytoplasm as appears from the next sexual generation. Thus, while $mt^{XIII}$ is recessive with regard to its effect on mating type potentialities, it is dominant with respect to its effect on the mating type-determining state of the cytoplasm.—(Supported by a predoctoral fellowship from the National Cancer Institute, and partially by a grant to T. M. Sonneborn, Indiana University, from the Atomic Energy Commission.)

Tejada, C., M. Sanchez, M. A. Guzman, N. S. Scrimshaw and E. Bregni, Instituto de Nutrición de Centro América y Panamá and Instituto Indigenista de Guatemala, Guatemala, C. A.: *Blood groups in Mayan population.*—The large Mayan Indian population of Guatemala is classified by the 21 native languages still in use which comprise seven major groups. Within each major language group, ten persons from each of four communities were studied. Among the 120 persons examined to date, the following phenotype frequencies were encountered: MMS .333, MNS .225, MNs .200, MMs .166, NNS .041, NNs. 033; P+ .708, P− .291; Kell + (K+) 0.000, K− 1.000; Kidd (80 cases) + ($Jk^a$+) .700; $Jk^a$− .300; Duffy + ($Fy^a$+) .816, $Fy^a$− .183; Diego + ($Di^a$+) .300, $Di^a$− 0.700. For the 40 in whom the A-B-O and Rh systems were determined, frequencies were as follows: A, B and AB .000, 0 1.000; CDe/CDe .425, CDE/cde .200, cDE/cDE .175, CDE/CDE .100, CDe/cde .050, CDE/CDe .025, cDe/cDE .025. The phenotypes found with the greatest frequency were 0, MMS, CDe/CDe. The prevalence of the $Di^a$+ is one of the highest reported.

Ting, Y. C., Harvard University, Cambridge, Mass.: *Meiosis in triploid maize-teosinte hybrids.*—In a population of 22 plants of the backcross hybrid (Huixta teosinte × Wilbur's flint) × Wilbur's flint, two plants proved to be triploids. The high frequency of triploids is attributed to the nuclear disturbance following hybridization between maize and teosinte. The production of an egg nucleus with unreduced chromosome number is the probable mechanism by which the triploid plants were produced.—Pachytene chromosome associations in these triploid plants appeared to be 2-by-2 instead of 3-by-3. At diakinesis, univalents averaging 1.5 per sporocyte, bivalents averaging 1.2 per sporocyte, and trivalents averaging 8.6, were present. Univalents at metaphase 1, and laggards (as many as eight in a single sporocyte) at anaphase 1, were observed. Precocious division of lagging dyads was frequently found. Fusion of two nucleoli, if by chance present, at prophase 2 always occurred, but fusion of nucleolar organizers was never observed. At metaphase 2 and anaphase 2, lagging chromosomes from one to four were sometimes present. However, micronuclei were only occasionally formed at quartet stage.

Trasler, Daphne G., McGill University, Montreal, Canada: *The influence of uterine position and other factors on the occurrence of spontaneous cleft lip in mice.*—It has been found that within the A/Jax strain embryos in the uterine position nearest the ovary develop spontaneous cleft lip significantly more often than those in other positions in the uterus. Maternal weight has also been found to influence the frequency of cleft lip. Mothers weighing over 21 gm have significantly lower numbers of cleft lip offspring than those weighing under 21 gm. The data supporting these conclusions will be presented and discussed, and other data relating to uterine position effects and maternal weight influence on the embryo will be presented.

Turner, J. Howard, and Eldon J. Garner, Utah State University, Logan, Utah: *Effects of copper and iron salts, on viability and abnormal growths in Drosophila melanogaster.*—Copper and iron salts were added to the medium of six laboratory stocks. First instar larvae were

collected from laying dishes and transferred to test and control media. Differential viability and phenotypic expression at different concentrations were evaluated. The results showed that increases in the level of copper and iron were accompanied by: (1) a linear increase in mortality response in both larval and pupal stages; this response exhibited a strong quadratic trend within the range of the chosen treatment levels; (2) increased duration of larval development; (3) striking differences in mortality among the tested stocks. Tumorous head and melanotic tumor stocks, when compared with wild stocks, displayed significantly higher mortality responses. Flies of one stock ($tu^{150}$) had no visible eye abnormality but, following treatment with copper sulfate, about 17% expressed a trait similar to the mutant eyeless. Flies showing the new trait were inbred and maintained on a medium with no added copper. Some 94% of the second generation progeny expressed the trait. Through inbreeding and selection in the next four generations a stock was developed in which nearly 100% expressed the trait. The new stock has now been maintained for 30 generations on the standard medium with no loss in expression. The experiment was repeated and similar results were obtained. Copper sulfate was then injected directly into the bodies of $tu^{150}$ flies. Some of the progeny of the injected parents showed the same phenotype as the flies resulting from the copper feeding experiments. Again the trait was maintained through inbreeding and selection and a line with nearly 100% expression was developed on the standard medium. (Supported by the American Cancer Society and Damon Runyon Memorial Fund.)

TUVESON, R. W., and E. D. GARBER, University of Chicago, Chicago, Ill.: *The virulence of biochemical mutants of Fusarium oxysporum f. pisi.*—Nine varieties of garden pea *(Pisum sativum)* were inoculated with biochemical mutants of *F. oxysporum f. pisi*, the fungal incitant of pea wilt, requiring specific amino acids, purines, vitamins, or growth factors. The mutants displayed a pattern of virulence and avirulence. No mutant was virulent for all varieties; some mutants were avirulent for all varieties. These and other observations suggest that the biochemical constitution of plants of these varieties differ, at least on a quantitative basis. (Aided by the Office of Naval Research and the Abbott Memorial Fund, University of Chicago.)

WEAVER, ELLEN C., University of California, Berkeley, California: *Quantitative inheritance of frequency of somatic crossing over in Drosophila melanogaster.*—Two unrelated stocks of *D. melanogaster* were selected without any prior knowledge of their tendencies towards mosaicism. Somatic crossing over was measured in the X chromosome using the classic *y* and $sn^3$ system. It was found that the two stocks differed sharply and significantly in their incidence of mosiacism, one stock having an average of 0.7 spots per fly, the other 1.7 spots per fly. There was no difference between the two in mean spot size or in distribution of mutant patches.—Classes of flies resulting from $F_2$ segregations of crosses between the two stocks revealed that factors governing the frequency of X chromosome mitotic recombination occurred on all three major chromosomes, that is, the second, third and X itself. The recombination classes having the same genetic content as the parents duplicated the parental frequencies of spotting.—Constants for the contribution of each chromosome to the total mosaicism were determined. The expected values derived from these constants for each segregating class agreed very well with the experimental ones.—It is concluded that somatic crossing over is a process under the precise control of genetic factors governing the frequency of its occurrence.

WEBBER, B. B., Yale University, New Haven, Conn.: *Comparative complementation and genetic maps of the hist-3 locus in Neurospora crassa.*—Combined biochemical and genetic studies have indicated that allelic mutants at the *hist-3* locus fall into three basic categories, as follows: sub-group (1) strains lacking histidinol dehydrogenase activity; sub-group (2) strains lacking activity for an as yet unidentified enzyme involved in an early reaction (before imidazole glycerol phosphate) in histidine biosynthesis; and sub-group (3) strains lacking both enzyme activities.—Further detailed studies of a series of *hist-3* mutants have led to the development of both a heterocaryon complementation map and a genetic map of this locus. The complementa-

tion map comprises eleven functional units (cistrons) and contains at one end, the subgroup 1 mutants (which accumulate histidinol in growth medium), while sub-group 2 and 3 strains (non-accumulators of histidinol) extend into or are entirely localized in the opposite end of the complementation map. Strains characterized by slight histidinol accumulation (which lack histidinol dehydrogenase activity and presumably possess only partial activity for the early enzyme) extend from the subgroup 1 region into an intermediate region.—Interallelic crosses of marked *hist-3* strains have yielded histidine-independent progeny in frequencies (all less than 0.02% of viable ascospores plated) which permit construction of a preliminary genetic map of the *hist-3* locus. The genetic map is generally comparable to the complementation map in that subgroup 1 strains tend to be located at one end of the genetic map and subgroup 2 and 3 strains at the other end, with weak accumulators intermediate in position. Current evidence is discussed which favors the concept of a single continuous *hist-3 locus*, divisible into three regions in which mutants in the three subgroups are located.

WEINBERG, ROGER, (Introduced by C. C. LI), University of Pittsburgh, Pittsburgh, Pa.: *Gene transfer in one mating type of Escherichia coli.*—The Hfr Hayes mating type of *Escherichia coli*, strain K-12, transfers a segment of its genome to recipient F- strains with high frequency (2% to 25%) in an oriented manner. Frequency of transfer is defined as recombinants per input Hfr, with an excess of F- recipients. Some markers are transferred at an earlier time than others, and transfer of early markers is seldom accompanied by cotransfer of later markers, while transfer of late markers is usually accompanied by cotransfer of early markers (JACOB, WOLLMAN, and HAYES 1956). This will be referred to as oriented linkage, and is accompanied by a decrease in transfer frequency for late markers. JACOB and WOLLMAN postulate a rupture region in which the chromosome breaks while being transferred. Markers past the rupture region are transferred with low frequency ($10^{-4}$%). We have studied markers located between the high frequency and low frequency regions, and it has been found that the loss of transfer frequency is gradual, not sudden, and that an oriented linkage relationship is maintained at least until cystine (*cys*), a marker showing 0.53% transfer frequency. The new markers studied, tryptophan (*try*), histidine (*hi*), and *cys* were located on the general linkage map of *Escherichia coli* as follows: galactose (*gal*), prophage lambda (*lambda*), *try*, *hi*, *cys*, streptomycin (*stm*), methionine (*me*). There is decreasing frequency of transfer from *gal* to *me*.

WEINMAN, ANTONIA A., and W. H. GABELMAN, University of Wisconsin, Madison, Wis.: *Variations in cytoplasmic sterility of carrot as measured by tetrad segregation in partially fertile plants.*—A partially fertile carrot plant was found, which shed pollen in tetrads; after self-pollination the progeny segregated for tetrad plants and normal plants (nontetrads). Pollengrains stained with aceto-carmine were considered to be viable, the nonstaining grains aborted.—Tetrads with all five possible combinations of viable and aborted pollen grains occurred. The frequency of the five classes of tetrads vary rather consistently with changes in fertility level, indicating a characteristic relationship. A comparison of the data with various mathematical models will be presented.

WOLFE, H. G., and J. A. WEIR, University of Kansas, Lawrence, Kansas: *Correlated responses to selection for blood-pH in the mouse.*—Since blood-pH is a complex phenotype it seems worthwhile to isolate contributing factors. Materials consist of an outbred line, T, and two lines, TH and TL, derived by individual selection with minimal inbreeding. Mean blood-pH values at the sixth generation are: Th — 7.557 ± .0073, T — 7.527 ± .0061, TL — 7.498 ± .0056. Selection responses have indicated control by a few loci with major effects. $F_1$ progeny from $S_4$ parents were intermediate between parental lines. Data from $F_2$ and BC progeny from $S_5$ parents were typical for a quantitative character.—Significant departures from equality of sexes, noted for earlier generations, have persisted.—Behavioral differences may influence blood pH by affecting changes in metabolic rate. Empirical observations led to the discovery that mice (TH, T, TL, $F_1$, $F_2$, BC) which were randomized, six to a cage, were subsequently caught for pH testing in a

non random order. For the $S_6$ generation, mice were randomized as for $S_4$ and $S_5$, but were segregated, one to a cage, for pH testing. A frequency distribution of sampling order of mice from each cage, prior to segregation for testing, showed a consistent strain-order effect in the predicted direction. Experiments to measure thyroid activity are in progress.

WOLFF, SHELDON, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Chromosome breakage and rejoining in irradiated sperm of Drosophila.*—Studies on the effect of oxygen to increase the numbers of chromosomal rearrangements induced by X-rays have led to the conclusion that in plant chromosomes oxygen affects the numbers of breaks induced and also the rejoining of these breaks. Studying the loss of X or Y chromosomes from irradiated Drosophila sperm, LUNING *et al.* (Hereditas 1957), however, have concluded from experiments performed in air and in nitrogen that only rejoining and not breakage was being affected. Since Drosophila sperm are relatively anoxic in air these latter experiments have now been repeated in nitrogen, air, and also in oxygen. It has been found that if the radiation is administered in oxygen so that the sperm are not anoxic, then as with plant chromosomes, an effect of oxygen is found both on breakage and on the rejoining of breaks.

WOLFSBERG, MARILYN F., Brookhaven National Laboratory, Upton, N.Y.: *The effect of 2,4 dinitrophenol upon $P^{32}$ loss in adult Drosophila pseudoobscura females.*—Newly emerged females fed for seven to ten days on either a standard cornmeal-molasses-agar control medium or one containing $5 \times 10^{-4}$M DNP, each labeled with $H_3P^{32}O_4$. They were then removed to unlabeled control or DNP food. The loss in radioactivity of individual females was followed for 14 days.—$P^{32}$ loss falls into two phases, each of which follows a first order reaction law. Control females lose 80% of their radiophosphorus by means of a slow phase having a half-time of about ten days and 20% by means of a fast phase having a half-time of about 1.5 days. DNP poisoned females lose 90% of their $P^{32}$ by means of the slow phase. They accordingly lose phosphorus more slowly than control sibs. (Research carried out under the auspices of the U.S. Atomic Energy Commission.)

WOLFSBERG, MARILYN F., Brookhaven National Laboratory, Upton, N.Y. and Institute of Genetics, University of Copenhagen, Denmark: *The egg-laying rhythm of mated Drosophila pseudoobscura.*—Daily egg-laying records of 250 females were maintained for three weeks after eclosion. Flies oviposit initially between the third and fifth day. Bursts of egg-laying average 1.9 days; rest periods average 1.2 days. Flies differ markedly in eggs per burst. The first three bursts are higher than later bursts (in poor layers the first burst is generally highest) and the last burst is generally lowest. Twenty percent of the least fecund flies experience four bursts; over 70% of the more fecund flies do so. Thus, poor layers result from few eggs per burst and few bursts per experimental time.—Morphological changes in the ovary accompany functional changes. During egg-laying, the gonad contains mature eggs; during rest, it contains only pre-yolk and early volk stages. A hormonal regulation of the rhythm has therefore been postulated. (Research carried out under the auspices of the U. S. Atomic Energy Commission).

YANDERS, A. F., Argonne National Laboratory, Lemont, Ill.: *The effect of X-rays on sperm activity in Drosophila.*—Estimates of the rate at which mutations are produced in Drosophila by ionizing radiation are based in part on studies involving irradiation of mature sperm. Reliability of these estimates depends on the validity of several assumptions. One is the assumption that irradiation does not influence the behavior of sperm, and that all sperm, affected and non-affected, are equally likely to participate in fertilization. This may not be true. Sperm activity is difficult to evaluate, but one aspect that can be measured is successful postcopulatory migration of sperm from the vagina to the ventral receptacle. A system of scoring the fullness of excised receptacles was used to estimate the relative activity of irradiated and nonirradiated sperm. Using this method, the degree of successful insemination produced by Oregon-R males mated

immediately after irradiation at doses as low as 2,500r is measurably less than the control rate. If the males are held 24 hours or longer before mating, some recovery occurs; recovery may be complete at doses below 10,000r.—This finding has a direct bearing on dominant lethal studies. KAPLAN (1958) has shown that unfertilized eggs may contribute significantly to the dominant lethality rate as measured by hatching failures. Irradiation may contribute to fertilization failure by (1) reducing the activity of individual sperm, and (2) lowering the number of sperm stored by a laying female. (Work performed under auspices of U. S. Atomic Energy Commission.)

YERGANIAN, GEORGE, and MARY J. LEONARD, The Children's Cancer Research Foundation and Harvard Medical School, Boston, Massachusetts.: *A stable mammalian in vitro system having maximum proliferating ability*.—Fibroblast-like cells derived from 18-day old embryos of the inbred Chinese hamster undergo rapid proliferation for many generations prior to experiencing heteroploid alterations (23–24 chromosomes). During this period, the availability of 95% of the cells in the classic diploid state ($2n = 22$) facilitates numerous experimental procedures. Spontaneous chromosome breakage rarely exceeds 0.04% during the early phases of growth, whereas the frequency will rise to 0.25% breaks per 100 cells when aneuploidy occurs or following some 15 passages.—The X and Y chromosomes are rapidly visualized by their extensive display of differentially spiralled "block" heterochromatin in the mitotic metaphase (as contrasted to other mammalian systems) thereby encouraging mixed population studies involving sex chromosomes as markers to determine the selective advantages of distinctly new chromosome rearrangements in certain sublines. Whenever necessary, republication of trials is facilitated by employing pregnant inbred littermates or siblings, thereby continuing the genetic background of the tissues. Point mutations and heteroploid alterations, which one must be prepared to offset with cloning procedures, are conveniently bypassed by the use of this replicable inbred embryonal fibroblast system.—Quasidiploidy and aneuploidy provide interesting variants. Similarly, subdiploid cells of the Chinese hamster, derived following the introduction of the SE mouse polyoma virus in newborn, provide unique monosomic relationships for single cell cloning, exhibit rare mutations, and display a phenomenally high proliferative ability. (This work is supported in part by grant #293C from the Damon Runyon Fund and grant #G-2829 from the National Science Foundation).

# MATERNAL EFFECT OF ma-l$^+$ ON XANTHINE DEHYDROGENASE OF DROSOPHILA MELANOGASTER[1]

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PREVIOUS work has indicated that there are at least two loci (*ma-l*$^+$ and *ry*$^+$)* in *Drosophila melanogaster* capable of mutating independently to produce a deficiency of xanthine dehydrogenase (FORREST, GLASSMAN and MITCHELL 1956). Some results of this deficiency are accumulations of the substrates (hypoxanthine and 2-amino-4-hydroxypteridine) of this enzyme, and a lack of the products (uric acid and isoxanthopterin) formed from these compounds (MITCHELL, GLASSMAN and HADORN 1959). In addition, the red eye pigment of these flies is diminished so that the color of the eye is dark red-brown; however, the direct relation between the enzyme deficiency and the eye color is obscure at present. The enzyme from wild type adults has been purified with charcoal, ammonium sulphate, and calcium phosphate gel, but treatment of the mutant extracts with this procedure has failed to elicit enzymatic activity indicating that the deficiency in the mutants is not due to simple inhibitors (GLASSMAN and MITCHELL 1958). The present report concerns a maternal effect associated with *ma-l*$^+$, which is not dependent on the presence of *ry*$^+$. A preliminary report of this investigation has appeared (GLASSMAN, HUBBY and MITCHELL 1958).

## MATERIALS AND METHODS

Unless stated otherwise, all procedures followed those reported previously (GLASSMAN and MITCHELL 1959). The enzyme was assayed in various strains as follows: 100 adult flies, one day old, were homogenized in 1.0 ml of 0.1 *M* Tris buffer, pH 8. Two to five mg of Norit-A were added, and after ten minutes the mixture was centrifuged to remove the charcoal. The colorless supernate (0.5 ml) was added to an assay mixture containing 0.5 ml buffer; 0.01 ml $10^{-3}$ M 2-amino-4-hydroxypteridine; and 0.01 ml $10^{-3}$ M methylene blue, and the reaction was followed photofluormetrically as described previously (GLASSMAN and MITCHELL

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* The following abbreviations for various mutants are used (BRIDGES and BREHME 1944): *Bx*$^3$ = Beadex wing; *cu* = curved wing; *f* = forked bristle; *m* = miniature wing; *ma-l* = maroon-like eye color; *ry* = rosy eye color; *ss* = spineless bristle; *st* = scarlet eye color; *TM1* = third chromosome balancer (LEWIS, pers. comm.); *th* = thread anthenae; *y* = yellow body color; *y f*: = is an attached-X chromosome containing *y* and *f*.

1959), except that the quinine standard was set at 100 instead of 30 to increase sensitivity.

Chromatographic procedures were carried out as previously described (HADORN and MITCHELL 1951; GLASSMAN and MITCHELL 1959). Analysis of single pupae and flies was accomplished by squashing directly on the chromatography paper, but extracts were also used as follows: 50 flies were placed in a small homogenizer with 0.2 ml of distilled water or 1 percent $NH_4OH$, and subjected to a boiling water bath for a few minutes. They were then homogenized and again heated in a water bath. The mixture was then centrifuged, and the resulting supernate was applied as a one cm streak at the starting line of the paper chromatogram.

The combination of *st* with *ma-l* or *ry* produces orange eyes. This greatly facilitates the separation of *ma-l*$^+$ from *ma-l*, and *ry*$^+$ from *ry*, and for this reason the stocks used in this investigation contained *st*.

## RESULTS

Progeny analysis of reciprocal crosses between *ma-l* and wild type revealed that *ma-l* is a typical sex-linked recessive as reported by OLIVER (see BRIDGES and BREHME). However, when the $F_1$ *ma-l*/*ma-l*$^+$ females are crossed to *ma-l* males, the expected *ma-l* phenotype does not appear even though half the flies are genetically *ma-l* as revealed by genetic markers and progeny tests. The effect is also obtained when attached-X females (*y f*: = ; *st*) are crossed to *ma-l* ; *st* males, and most of the analysis was accomplished with this type of cross.

Since *ma-l* is probably nonautonomous (GLASSMAN 1958), one explanation might be that the wild type progeny excrete products which are capable of affecting their *ma-l* sibs during larval development. However, this is disproved by the cross, *ma-l*/*ma-l* × wild type, which produces normal *ma-l* males, and also by further experiments in which attached-X females (*y f*: =; *st*, mated to *ma-l*$^+$ males) and *ma-l* females (*y ec ma-l* ; *st* females mated to males of their own genotype) were allowed simultaneously to lay eggs in the absence of the males in the same bottle, simulating the conditions found in the cross between attached-X females and *ma-l* males. All *y ec ma-l* ; *st* offspring had *ma-l* eyes indicating that no cross-feeding occurs between wild type flies and *ma-l*.

The progeny from maternally-affected males and females have the normal ma-l phenotype, and, therefore, the maternal effect is due to a "predetermination" of the egg and is not due to self-reproducing cytoplasmic particles. Additional tests indicated that the presence of other genes (*al*, *B*, *Bx*$^3$, *ec*, *f*, *fu*, *m*, *m*$^D$, *pol*, *ptg*$^3$, *sn*$^3$, *st*, *wy*, *y* (see BRIDGES and BREHME).) did not alter this effect. The usual interpretation for this type of phenomenon is that the *ma-l*$^+$ females are passing a substance to their progeny through the egg. For convenience, we shall designate this material as "*x*."

### *Biochemistry*

Paper chromatography revealed significant differences between the normal *ma-l* males and the maternally-affected ones. First, the maternally-affected males

have increased amounts of the red pigment, and their eye color resembles the wild type exactly. Second, traces of uric acid and isoxanthopterin (the enzyme reaction products) are present indicating that xanthine dehydrogenase occurs in these flies, and, indeed, small amounts of enzymatic activity were found when the maternally-affected flies were assayed (Figure 1). This small change in

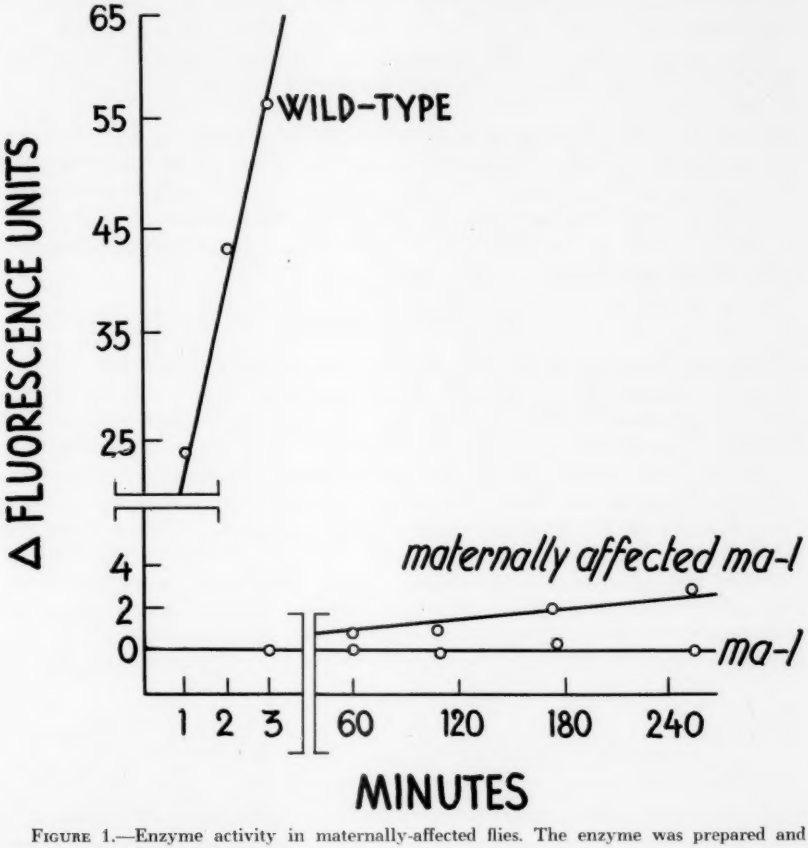


FIGURE 1.—Enzyme activity in maternally-affected flies. The enzyme was prepared and assayed as described under Methods.

fluorescence in extracts of maternally-affected *ma-l* was shown to be due to the formation of isoxanthopterin as follows: one gram of maternally-affected *ma-l* males, less than one-day old, was homogenized in 2 ml 0.1 M Tris buffer, pH 8.0, and the solution was centrifuged at 30,000 × g for 15 minutes. Charcoal (Norite-A) was added to the supernatant solution in 100 mg portions until all traces of eye pigment were removed. To 0.3 ml of the colorless extract were added 0.01 ml

of $10^{-3}$ M 2-amino-4-hydroxypteridine and 0.01 ml of $10^{-3}$ M methylene blue. The mixture (0.1 ml) was applied as a one cm streak for paper chromatography before and after a three hour incubation at 25°C. The chromatogram was developed with propanol: one percent ammonia (2:1) for two hours. The use of an ultraviolet lamp emitting at 3600 Å revealed traces of isoxanthopterin after incubation, but none before. Control extracts of normal *ma-l* did not produce any isoxanthopterin.

Visual inspection of the chromatograms of maternally-affected flies reveals that the amounts of the enzyme substrates (hypoxanthine and 2-amino-4-hydroxypteridine) present are variable but lie between the amounts found in the usual *ma-l* and the wild type male. However, when the spot containing the 2-amino-4-hydroxypteridine is eluted from the paper and the amount of fluorescence of the eluate determined, it appears that more fluorescence is present in the maternally-affected flies than in *ma-l* or wild type (HUBBY and FORREST unpublished). These results are being pursued further, and may indicate the existence of another compound involved in this system.

### *Effect of environment*

Only *ma-l* flies which emerge in the first six to eight days after the first flies appear are maternally-affected; after this time a short period occurs (one to two days) during which the flies emerging have intermediate eye colors between *ma-l* and wild type, but later none is maternally-affected and all have the typical ma-l phenotype. This reversal could be due to a depletion of "*x*" in the female parent, or to a change in the condition of the culture. To test these alternatives, *y f*:=; *st* females were mated to *m ma-l* ; *st* males (Table 1), and transferred periodically to new bottles. Each successive bottle was then examined daily to determine the first day that males which were not maternally affected appeared. The results show that the time of disappearance of the maternal effect is the same regardless of the age of the female. Thus, it seems that the *ma-l*$^+$ females are capable of producing "*x*" throughout their lives, and that the decline in the maternal effect is

TABLE 1

*The effect of age* vs. *culture conditions on the maternal effect of* ma-l$^+$

| Transfer | Number of days from previous transfer | Number of days between transfer and the disappearance of the maternal effect |
|---|---|---|
| 1 | . | 16 |
| 2 | 5 | 15 |
| 3 | 6 | 16 |
| 4 | 6 | 17 |
| 5 | 5 | 17 |
| 6 | 7 | 15 |
| 7 | 6 | 17 |

Ten pairs of the mating *yf*:= ; *st* females × *m ma-l*; *st* males were successively transferred to fresh bottles at five to seven day intervals. The bottles were examined daily in order to determine the first day that male progeny which are not maternally affected appeared.

due to a change in the culture environment. This interpretation received further support when ten pairs of the mating, attached-X females × *f Bx*$^3$ *ma-l* males,* were added to food bottles in which attached-X females (mated to *m ma-l males**) had been allowed to lay eggs for seven days. The bottles were then examined daily in order to determine the time at which the maternal effect diminished for the *m ma-l* and *f Bx*$^3$ *ma-l* males, respectively. The *m ma-l* males showed the usual pattern, i.e., these males were maternally affected until five to six days after the first flies emerged; however, during the time the *f Bx*$^3$ *ma-l* males developed the critical change had occurred in the culture, and none of these flies was maternally affected.

The nature of the change is not known. It may be due to the depletion of a dietary factor necessary for the maternal effect, or it may be due to the accumulation of metabolic substances which can inhibit the small amounts of enzyme present in the maternally-affected flies. Experiments designed to test these alternatives are in progress.

### *Effect of* ry

The mutant *ry* is located on the 3rd chromosome at 51±. When females heterozygous for *ry*$^1$ (*th st cu ry*$^1$ *ss* / *TM1*) are crossed to *ry*$^1$ males (*th st cu ry*$^1$ *ss* or *th st cu ry*$^1$ *ss* / *TM1*), the non-*TM1* progeny are phenotypically normal *ry*, indicating that *ry*$^1$ is not maternally affected by *ry*$^+$. A similar result was obtained using *ry*$^2$. Since both *ry* and *ma-l* are deficient in xanthine dehydrogenase, one might expect that *ry* / *ry* might negate the maternal effect of *ma-l*$^+$. Accordingly, attached-X females containing *st ry*$^1$ or *st ry*$^2$ (i.e., *y f*: = ; *st ry*) were mated to *m ma-l* ; *st* males. The result was unexpected since the maternal effect of *ma-l*$^+$ was still manifested in the *m ma-l* ; *st ry* / *st*$^+$ *ry*$^+$ male progeny. Thus, the maternal effect of *ma-l*$^+$ does not depend upon the simultaneous presence of *ry*$^+$. It should be noted that mating the above *y f*: = ; *st ry*$^2$ females to males containing both *ma-l* and *ry* (*ma-l* ; *st ry*$^2$) produced *ma-l* ; *st ry* males which do not exhibit a maternal effect, since they are homozygous for *ry*.

## DISCUSSION

Maternal effects of the type associated with *ma-l*$^+$ occur in many organisms. These are characterized by a "predetermination" of the egg cytoplasm by the physiological condition of the female parent so that the progeny reflect her genetic constitution, even though they may have a different genotype. This effect does not persist beyond one generation, since maternally-affected organisms have progeny which are typically mutant. This phenomenon is interpreted as being due to a substance which the female parent passes to her progeny; the lack of persistence beyond one generation is taken to mean that this compound is not synthesized in the maternally-affected mutant, and is diluted out or used up during protoplasmic growth. In the case of *ma-l*$^+$, this contribution from the female parent is designated as "*x*."

* The crosses were *y f*:=; *st* × *f Bx*$^3$ *ma-l*; *st* and *y f*:=; *st* × *m ma-l*; *st*.

An example of this type of maternal influence is associated with sinistral and dextral coiling in snails (BOYCOTT *et al.* 1930), some egg color mutants of Bombyx (KIKKAWA 1957), the *a* mutant of Ephestia (CASPARI 1933), the light eye color mutant (BEADLE 1937) and the vermilion eye color mutant (GRAF 1957) of *D. melanogaster*, and many others. These maternal effects are associated with processes initiating in the egg or in early development, whereas the effect seems to be much later with *ma-l*$^+$; indeed, "*x*" apparently persists to the adult stage where we can observe a wild type eye color and traces of xanthine dehydrogenase and its reaction products. This would tend to eliminate the idea that "*x*" is a simple substance such as an enzyme activator or cofactor, since such compounds which are not being resynthesized would probably be depleted prior to the adult stage.

On the other hand, "*x*" may represent the enzyme, particularly since there is approximately only 1/1000 the enzyme activity in maternally-affected *ma-l* males than there is in their female parent. Comparison of the weight of the adult female (approximately 0.8 mg) and the egg (about 0.008 mg) indicates that 1/100 of the enzyme present in the adult female can be passed to the egg, if we assume that the enzyme is distributed equally throughout the adult and the egg. This may not be true since the egg contains considerable yolk, which may not carry the enzyme. In any case, the small amount of enzyme present in the adult would be consistent with the idea that it is "*x*." However, since females which are homozygous for *ry* (*y f*: = ; *st ry*$^2$) and which are therefore deficient in xanthine dehydrogenase, can still exert a maternal effect on *ma-l* progeny, it is difficult to see how this enzyme could be "*x*." Another alternative is that "*x*" is a component of the enzyme-forming system, which might be stable, and which could overcome possible cytoplasmic dilution by producing many enzyme mole-

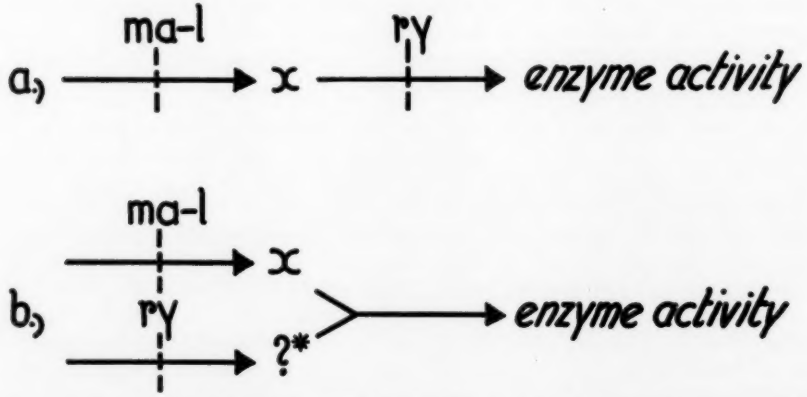


FIGURE 2.—Possible alternatives to explain the effect of *ma-l* and *ry* on *x*. This compound is not passed into the egg, whereas *x* is.

cules. Data on this point are lacking, however, and it is difficult to rule out any possibility.

The question arises as to why *ry* flies cannot respond to "*x*" in the egg. Since *ma-l*$^+$ ; *ry* females can exert a maternal effect on *ma-l*, it is obvious that *ry* flies can synthesize "*x*." Thus, it would appear that *ry* flies cannot respond to maternal influences because they are blocked in the utilization of "*x*." On the other hand, the maternal effect on *ma-l* most likely occurs because these flies can utilize "*x*" if it is present in the egg. These data suggest that a sequential relationship may exist whereby *ma-l* is blocked in the synthesis of "*x*," and *ry* is blocked in its utilization; a simple scheme (Figure 2a) can account for these data, but other equally likely pathways can also be applied (Figure 2b). It is of interest that the only other difference between *ma-l* and *ry* which has been found concerns the presence of a substance which can cross-react with the antibody to xanthine dehydrogenase. This substance is present in *ma-l* and deficient in *ry* (GLASSMAN and MITCHELL 1958). Its significance is not yet known.

## SUMMARY

Previous work has shown that two eye color mutants of *Drosophila melanogaster* (*ry* and *ma-l*) are deficient in xanthine dehydrogenase. The present study concerns a maternal effect which *ma-l*$^+$ / *ma-l* females have upon their *ma-l* progeny. It is of interest that *ry*$^+$ / *ry* females do not exert a similar effect on *ry*. Biochemical studies have shown that in addition to increased amounts of red eye pigment, the maternally-affected *ma-l* flies have traces of isoxanthopterin and uric acid (the enzyme products), as well as traces of the enzyme itself. This case is unusual, since most maternal effects of this type usually affect processes initiating in the egg or in early development, while the effect here seems to be much later in development.

However, only flies which emerge in the first six to ten days of hatching show this effect; following this time the adults which emerge in the bottle have the typical *ma-l* eye color. This is due to a change in the culture and not to the age of the female parent, since old females transferred periodically to new bottles will also have maternally-affected progeny in each new bottle until six to ten days after the first flies emerge. The nature of this change is not known.

The data suggest that the *ma-l*$^+$ / *ma-l* females are passing a substance to their progeny in the egg cytoplasm. This substance is not made in *ma-l* flies, but can be utilized by this mutant; hence, the maternal effect. On the other hand, since *ma-l*$^+$ / *ma-l* ; *ry* females can exert a maternal effect on *ma-l*, it is evident that *ry* can synthesize this compound and pass it to the egg; its utilization is probably blocked in this mutant. This suggests that a sequential relationship may exist between the reactions blocked by these mutants.

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# SELECTIVE FERTILIZATION IN LIMA BEANS

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SELECTIVE fertilization or incompatibility has been reported to exist in many plant species. The usual reaction which results in these phenomena is one between the diploid style and the haploid pollen tube so that selective fertilization occurs. Selective fertilization in lima beans appears to be similar to that reported in maize by EMERSON (1925) and MANGELSDORF and JONES (1926) where the effect of the dominant gametophyte factor *Ga* is operative only when *ga* and *Ga* pollen are competing on stylar tissue which is *Ga/Ga* or *Ga/ga*. Under these conditions pollen gametes with the recessive *ga* factor fail to effect fertilization, or effect only a small percentage of the fertilizations. In a species such as lima beans where controlled pollination is restrictive in producing large backcross populations, such a gametophyte factor can be detected only when it is linked with a qualitative factor in progenies of self-fertilized heterozygous plants. Qualitative factors when linked with the gametophyte factor will be detected by having either a deficient or excess class depending on whether the linkage is in the repulsion or coupling phase.

## MATERIALS AND METHODS

### *Determinate vs. indeterminate plant habit of growth*

$F_2$ progenies segregating for the *D/d* gene were observed and recorded incidental to a lima bean breeding project at the University of Illinois, and it was noted that all of the progenies did not fit the expected 3 vine:1 bush ratio.

ALLARD (1953) reported that the determinate habit of growth of lima bean plants was inherited as a monogenic recessive. MACKIE and SMITH (1935) had observed other ratios, in addition to the usual 3 vine:1 bush in $F_2$ progenies, although no supporting data were given. ALLARD speculated on the possibility of genes at other loci for this character and on the basis of his data concluded that if they did occur their frequency must be low in the species.

One indeterminate parent plant in particular was associated with the aberrant ratios observed in Illinois. Twelve crosses had been made using this indeterminate plant (designated as G.F.B.) and the segregating $F_2$ progenies are shown in Table 1. In all crosses G.F.B. was used as the male parent and the $F_1$ progeny were all indeterminate. Three of the 12 $F_2$ progenies when tested for the 3:1 fit, had probabilities of less than .01, and an additional five had probabilities of less than .20. The pooled data was tested and a chi-square ($\chi^2$) of 47.176 for the 3:1 fit was calculated, having a probability value of less than .01. The data were then

TABLE 1

*$F_2$ progenies from plant G.F.B. used as the male parent. The aberrant 3:1 ratios for the segregating* D/d *genes are shown indicating selective fertilization. Recombination percentages for the* D-Ga *loci are calculated*

| Cross no. | Year grown | Observed D- | Observed dd | $\chi^2$(3:1) | P | Percent recombination between *D-Ga* loci |
|---|---|---|---|---|---|---|
| 1055–55 | 1955 | 117 | 67 | 13.356 | >.01 | 27.17± 7.30 |
| 1055–F | 1956 | 173 | 86 | 9.309 | >.01 | 34.36± 5.83 |
| 1055–GH | 1956 | 81 | 40 | 4.189 | .02–.05 | 33.88± 8.55 |
| 1059 | 1956 | 37 | 15 | .411 | .50–.70 | 42.31±12.56 |
| 1060 | 1956 | 175 | 69 | 1.399 | .20–.30 | 43.44± 5.77 |
| 1062 | 1956 | 97 | 41 | 1.633 | .20–.30 | 40.58± 7.05 |
| 1060(24–16) | 1957 | 322 | 133 | 3.937 | .02–.05 | 41.54± 4.26 |
| 1104–A | 1957 | 105 | 43 | 1.180 | .20–.30 | 41.89± 7.46 |
| 1106 | 1957 | 123 | 55 | 2.901 | .05–.10 | 38.20± 6.83 |
| 1061 | 1957 | 429 | 172 | 3.853 | .02–.05 | 42.76± 3.96 |
| 1061–G.H. | 1957 | 117 | 49 | 1.807 | .10–.20 | 40.96± 7.08 |
| 1104–B | 1958 | 408 | 180 | 9.877 | >.01 | 38.77± 3.02 |
| (Pooled) | | 2,184 | 950 | 47.176 | >.01 | 39.37± 1.64 |
| Homogeneity | | | | 6.676 | .80–.90 | |

tested for homogeneity and the chi-square ($\chi^2$) of 6.676 with 11 degrees of freedom gave a probability value of .80-.90. The deficient class was the indeterminate type and the data were homogeneous.

## RESULTS

### *Linkage of* D/Ga *loci*

Based on an hypothesis as reported by EMERSON (1925) and MANGELSDORF and JONES (1926) for maize it was assumed that plant G.F.B. was homozygous for a recessive gametophyte factor that failed to effect fertilization when in competition with a dominant gametophyte factor, and that this factor was linked with the *D* loci. The linkage measurement would be restricted to the male gametes even though crossing over was assumed to be equal in both sexes. Following the "Method of Maximum Likelihood" (MATHER 1951) the formula was derived which gave a linkage measurement and the standard error for such a measurement. The formula $p = \frac{a_1 - a_2}{n}$ gives the recombination value for repulsion linkage in a $F_2$ population where $a_1$ = dominant class, $a_2$ = recessive class and $n$ = total population. The standard error of the recombination value was calculated using the formula $S_p = \sqrt{\frac{(1-p)(1+p)}{n}}$ where $p$ = the recombination value.

The linkage values calculated for each cross and the polled data are shown in Table 1. For the pooled data the percentage of recombination between the *D* and

*Ga* loci was calculated to be 39.37 ± 1.64. A small population of the backcross *d/Ga d/Ga* × *D/ga d/Ga* was observed to segregate 15 *D*-:25 *dd*. This 15:25 ratio fits a 1:1 with a probability value of .10-.20. However, if we assume the *ga* pollen gametes as being nonfunctional and 40 percent recombination between the *D-Ga* loci then the expected ratio becomes 16:24 which is very near the observed 15:25. The calculated recombination value for this backcross population is 37.5 ± 7.65 percent. This backcross population, because of its small number, is not indicative of selective fertilization in itself but when considered in the light of the $F_2$ data it supports the hypothesis of a gametophytic factor causing selective fertilization.

The effect of the gametophyte factor was observed in a sample of $F_3$ families from cross #1061. Seventy-nine indeterminate plants were sampled from a field grown $F_2$ population. Twenty seeds from each plant which were assumed to be self pollinated were planted in the greenhouse and were observed as to whether they segregated determinate plants. If unrestricted fertilization occurred in the $F_2$ plants a ratio of 1 nonsegregating to two segregating $F_3$ families should be expected. However, if selective fertilization occurred based on the proposed hypothesis, then the expected ratio should be two nonsegregating to five segregating $F_3$ families. The 79 $F_3$ families segregated 19 all indeterminate to 60 segregating determinate plants. This ratio of 19:60 when tested for an expected ratio of 1:2 had a chi-square value of 3.037 with a probability of .10-.05 and when tested for a 2:5 ratio the chi-square was reduced to .803 with a probability of .50-.30. Because of the closeness of a 1:2 and a 2:5 ratio the sample of 79 families was not large enough to disprove one of the ratios but based on the probability values the 2:5 ratio was not improbable.

"Sixteen $F_3$ families from self pollinated heterozygous indeterminate $F_2$ plants were field tested in 1958 to determine if expected deviations from a 3:1 (indeterminate *vs.* determinate) ratio occurred. Based on the proposed hypothesis of a recessive gametophyte factor linked in the repulsion phase and having 40 percent recombination with the *D* locus, the $F_3$ families would be expected to segregate into a 7.7:5.8:2.5 ratio for segregation of 3:1; 7:3 and 4:1 indeterminate *vs.* determinate respectively. The 7:3 ratio indicates repulsion linkage, the 4:1 ratio indicates coupling linkage and the 3:1 ratio indicates homozygosity of the *Ga* factor. The data from the $F_3$ families are shown in Table 2. The size of the individual $F_3$ families was reduced due to unfavorable growing conditions and this prohibited the disproving of 3:1 ratios, but based on the smallest probability the 16 $F_3$ families segregated into a ratio of 6:7:3 which fits the expected 7.7:5.8:2.5 ratio having a probability value of .70. This 6:7:3 segregation is not statistically valid but nevertheless is indicative of the expected deviation based on the proposed hypothesis. The significant fact indicated by the $F_3$ data was the occurrence of results which seem to fit the 4:1 ratios indicating coupling phase linkage between the *D* and *Ga* loci."

### *Effect of* Ga *factor on other loci*

The observed aberrant ratios for indeterminate *vs.* determinate plant type may be due to factors other than selective fertilization as proposed here. In order to

TABLE 2

*Progenies from $F_3$ plants showing segregation of the indeterminate-determinate types into 3:1, 7:3 and 4:1 ratios*

| $F_2$ family | Observed | | Chi-square value and approximate probability for expected ratios of: | | | | | |
|---|---|---|---|---|---|---|---|---|
| number | Indet. | Det. | 3:1 | | 7:3 | | 4:1 | |
| 10 | 153 | 56 | 0.359 | .56 | 1.023 | .32 | 6.030 | .01 |
| 14 | 101 | 37 | 0.241 | .64 | 0.668 | .43 | 4.001 | .05 |
| 15 | 182 | 68 | 0.645 | .44 | 0.960 | .34 | 8.100 | >.01 |
| 18 | 83 | 31 | 0.292 | .61 | 0.427 | .52 | 3.686 | .05 |
| 22 | 90 | 27 | 0.231 | .65 | 2.029 | .16 | 0.692 | .42 |
| 25 | 152 | 57 | 0.567 | .46 | 0.740 | .41 | 6.909 | >.01 |
| Pooled | 761 | 276 | 1.443 | .23 | 5.657 | .02 | 28.362 | >.01 |
| 26 | 86 | 34 | 0.711 | .42 | 0.166 | .69 | 5.209 | .02 |
| 1 | 98 | 41 | 1.499 | .23 | 0.017 | .90 | 7.835 | >.01 |
| 13 | 279 | 110 | 2.228 | .15 | 0.550 | .47 | 16.659 | >.01 |
| 19 | 109 | 50 | 3.524 | .05 | 0.158 | .69 | 13.020 | >.01 |
| 20 | 316 | 120 | 1.480 | .23 | 1.274 | .27 | 15.422 | >.01 |
| 21 | 92 | 39 | 1.591 | .21 | 0.003 | .97 | 8.752 | >.01 |
| 23 | 109 | 45 | 1.464 | .23 | 0.044 | .84 | 8.184 | >.01 |
| Pooled | 1,089 | 439 | 11.340 | >.01 | 1.173 | .28 | 72.789 | >.01 |
| 9 | 145 | 36 | 2.521 | .12 | 8.810 | .01 | 0.001 | .98 |
| 24 | 142 | 35 | 2.578 | .12 | 8.814 | .01 | 0.006 | .97 |
| 27 | 99 | 25 | 1.548 | .22 | 5.716 | .02 | 0.002 | .98 |
| Pooled | 386 | 96 | 6.641 | >.01 | 23.334 | >.01 | .002 | .98 |

resolve this, the effect of the *Ga* locus was observed with respect to loci other than the *D* locus.

In lima beans the gene *Wl/wl* (willow leaf) governs leaflet shape. The homozygous dominant genotype produces a lanceolate leaflet, the heterozygous genotype produces an ovate-lanceolate leaflet and the homozygous recessive genotype produces an ovate leaflet (ALLARD 1953). The *Wl* locus is very closely linked with the *D* locus, having a calculated recombination value of 1.8 ± .4 percent. A cross between a California line L62 (*WlWldd*) and G.F.B. (*wlwlDD*) was made and the $F_2$ segregation of the *Wl* gene is shown in Table 3. The data did not fit a calculated 1:2:1 ratio, having a probability value of less than .01 indicating that selective fertilization occurred with respect to the *Wl* locus. The deficient class was the homozygous recessive. Assuming coupling linkage between the *Wl* and *Ga* loci the recombination percentage was calculated using the formula $p=\frac{a_2}{a_1+a_2}$, where $a_1$ is the homozygous dominant class, and $a_2$ the homozygous recessive class. The formula $Sp=\sqrt{\frac{2_p\,(1-p)}{n}}$ gives the standard error of $p$. The recom-

TABLE 3

*Goodness of fit for 1:2:1 and 3:5:2 ratios for the $F_2$ progeny of cross no. 1104* (Wl/Ga × wl/ga). *The 3:5:2 calculated ratio is based on 40 percent recombination between the* wl-Ga *loci*

| Genotype | Obs. no. | Expected ratios | |
|---|---|---|---|
| | | 1:2:1 | 3:5:2 |
| *WlWl* | 220 | 184 | 220.8 |
| *Wlwl* | 353 | 368 | 368.0 |
| *wlwl* | 163 | 184 | 147.2 |
| | 1:2:1 $\chi^2 = 10.359$ p = >.01 | | |
| | 3:5:2 $\chi^2 = 2.310$ p = .50–.30 | | |

bination value was calculated to be 42.56 ± 3.57. The recombination value between the *D* and *Ga* loci (repulsion linkage) for the same $F_2$ population was calculated to be 39.40 ± 3.39. Because of the close linkage between the *Wl* and *D* loci the $F_2$ population was not large enough to determine the spatial relation between these two loci. The effect of selective fertilization as demonstrated by the segregation of the *Wl*/*wl* gene indicates that the observed aberrant ratios for the *D*/*d* gene are not due to additional alleles for this character, but that the section of chromosome on which the *Wl* and *D* loci are located is affected by the action of another factor located on this chromosome.

In lima beans a dominant gene *R* conditions the dark red seed coat in the presence of the basic color factor *C*. (ALLARD 1953). ALLARD (1956) has shown that the *R* and *D* loci are linked. His data indicate a recombination value of .40 for these two factors. Although the data did not agree in supporting this recombination value, they demonstrated beyond reasonable doubt that these two loci were linked. Data from $F_2$ populations of four crosses involving the *R* and *D* loci were observed at Illinois and are shown in Table 4. The calculated recombination value for the pooled data was 22.11 ± 2.27 percent. The data were tested for homogeneity and a $\chi^2$ value of 3.476 with probability of .30-.50 indicated the data

TABLE 4

*$F_2$ progenies segregating for the* D/d *and* R/r *genes showing the linkage between these two loci. Two crosses were in the coupling phase and two in the repulsion phase. The recombination value for the combined data and the chi-square for homogenity of the data are calculated*

| Cross no. | a *D/R* | b *D/r* | c *d/R* | d *d/r* | Percent recombination between *D-R* loci | Score | Information | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|
| 1105 (C) | 38 | 6 | 5 | 10 | 20.3±5.9 | — 4.547377 | 256.119 | .081 |
| 1038 (C) | 163 | 24 | 21 | 44 | 18.6±2.7 | —31.041010 | 1,093.932 | .881 |
| 1106 (R) | 68 | 27 | 36 | 3 | 28.8±7.8 | +12.749360 | 150.750 | 1.078 |
| 1061 (R) | 191 | 81 | 111 | 8 | 26.5±4.7 | +24.977610 | 439.875 | 1.439 |
| | | | | | | + 2.138583 | 1,940.676 | 3.479 |
| | | | | | p = 22.11±2.27 ($\chi^2$ = 3.479—.002 = 3.477 p = .30–.50) | | | |

to be homogeneous. However, the genetic diversity of these data was not as great as in ALLARD's material which may partially account for the observed differences in the recombination values of 40 and 22 percent. The fact that the two loci are linked is quite evident. Since the percentage of recombinations for the *D—Ga* loci is approximately 40 and the *R—D* loci approximately 22, then the percentage of recombinations for the *R-Ga* loci should either be greater or less than the *D-Ga* loci depending upon the spatial relation of these three loci.

In two of the 12 crosses shown in Table 1 the *R* gene was segregating, and the $F_2$ data are shown in Table 5. These $F_2$ populations were also segregating mottle

TABLE 5

*$F_2$ progenies segregating for the* R/r *genes and calculated recombination values from coupling phase linkage between the* R-Ga *loci*

| Cross no. | Observed *R-* | Observed *rr* | $\chi^2$(3:1) | P | Percent recombination between *R-Ga* loci |
|---|---|---|---|---|---|
| 1106 | 104 | 30 | .4874 | .50 | 44.77±7.21 |
| 1061 | 302 | 89 | 1.0443 | .30 | 45.52±4.14 |
| Total | 406 | 119 | 1.5244 | .20–.30 | 45.33±3.65 |

patterns which reduced the classification of the entire population for the *R-:rr* genotype. The linkage between the *R-Ga* loci was in the coupling phase and was calculated using the formulae derived by the "Maximum Likelihood Method." The formula $p = \frac{2a_2}{n}$ gave the recombination value and the formula $S_p = \sqrt{\frac{(2-p)(p)}{n}}$ gave the standard error of the recombination value where $a_2$ = the recessive class, $n$ = the total population and $p$ = the recombination value. The recombination value for the pooled data (Table 5) was 45.33 ± 3.65 percent. This recombination value indicates that the *R* locus must be located on the opposite side of the *D* locus from the *Ga* locus. Interpolating from the table published by HALDANE (1919) the distance between the *R-D* loci is 24.2 centimorgans and between the *D-Ga* loci 51.1 centimorgans, which places the *R-Ga* distance at 75.3 centimorgans. The theoretical recombination value for the *R-Ga* loci is then interpolated to be 47.1 percent. This theoretical value does not differ significantly from the calculated value of 45.33 ± 3.65 percent.

## DISCUSSION

In calculating these recombination values the assumption was made that the *ga* gamete was completely inhibited from effecting fertilization. EMERSON (1934) estimated that an average of four percent of the fertilizations in maize were by *ga* gametes when in competition with *Ga* gametes on *Ga*-stylar tissue. If this is occurring in lima beans there is no direct evidence either for or against it. Indirect evidence that this might be occurring is the calculated recombination value

of 27.17 ± 7.30 percent for cross 1055—55 which was grown in 1955. This recombination value of 27.17 ± 7.30 which differs from the pooled value of 39.37 ± 1.64 may be the result of environmental inhibition of the *ga* gamete to a greater degree in 1955 than during 1956, 1957, or 1958.

SCHWARTZ (1950) reported a third allele at the *Ga* locus in maize which he named $Ga^s$. This factor was stronger in its action and the *ga* gametes seldom effected fertilization on $Ga^s/ga$ silks when in competition with $Ga^s$ gametes. Such a situation in lima beans could exist and might explain the reduced recombination values in the three 1055 crosses. These three crosses when grouped separately have a recombination value of 31.56 ± 3.99 percent, compared to the recombination value of 41.77 ± 2.04 percent for the eight remaining crosses. However it should be noted that in making the original crosses the *ga* gametes did effect fertilization on the *Ga/Ga* stylar tissue as long as there was no competition from *Ga* gametes. Furthermore the data from all crosses for observed vine:bush ratio were tested and found to be homogeneous with a probability value of .70–.80 (Table 1). In order to resolve the questions of the percentage of *ga* gametes that may effect fertilization and or the presence of additional alleles at the *Ga* locus, further recombination data for genes closely linked to the *Ga* locus is necessary.

## SUMMARY

Selective fertilization has been observed to occur in lima beans. The recessive *ga* pollen gametes are deficient in effecting fertilization on *Ga/ga* stylar tissue when in competition with *Ga* pollen gametes. The *D*, *Wl* and *R* loci are in the same linkage group with the *Ga* locus having 39.37 ± 1.64, 42.56 ± 3.57, and 45.33 ± 3.65 percent recombination with the *Ga* locus respectively. The *D* locus is between the *R* and *Ga* loci with 22.11 ± 2.27 percent recombination between the *R–D* loci. The *D*, *Wl* loci are very closely linked, 1.8 ± .4 percent recombination, and the spatial arrangement was not determined for these two loci. The linkage calculations involving the *Ga* locus were based on the assumption that the *ga* pollen gametes were completely inhibited from effecting fertilization on *Ga/ga* stylar tissue when in competition with *Ga* pollen gametes. The possibility of *ga* pollen gametes effecting fertilization when in competition with *Ga* pollen gametes or the possibility of additional alleles at the *Ga* locus which affect the amount of fertilizations was not resolved.

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# SILVERING (si) AND DIMINUTION (dm) OF COAT COLOR OF THE GUINEA PIG, AND MALE STERILITY OF THE WHITE OR NEAR-WHITE COMBINATION OF THESE[1,2]

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THE present paper is based on studies of the residual variability in the intensity of the colors due to the recognized genes in a largely self contained colony of guinea pigs. It is convenient to recognize seven major series of colors, each varying from a pale color, barely distinguishable from white to full intensity, primarily in accordance with the combination of *c* alleles, but secondarily because of the genes to be considered in this paper.

Empirical grades have been based on standard hair samples, chosen so that each grade is barely distinguishable from the preceding. One series of such standards has been used for the eumelanic colors (black, sepia, brown) and another for the phaeomelanic ones (yellow). Table 1 is taken from a previous paper to show the average grades and the standard deviations for the seven main series of colors (columns) and the various combinations of *c* alleles (WRIGHT 1949).

The standard deviations in Table 1 give an indication of the amount of residual variability in intensity but not fully. In describing these data, it was noted (WRIGHT 1949) "no dark eyed sepias from matings in this series of experiments have been excluded because their grades deviated unexpectedly much from the genotype which had to be assigned them. Thus a number of extremely light sepias of genotypes $c^dc^a$ and $c^rc^a$ (grades 7 to 9) segregated out and are included. Matings of such animals were, however, made in a different series of experiments designed to analyze extreme dilution and thus their progeny and descendants are not included here."

The same procedure was followed with unexpectedly light yellows, especially of genotypes $c^kc^r$, $c^kc^a$, $c^dc^r$, and $c^dc^a$ (grade 3 or less instead of the usual 4 or 5).

There were also unexpectedly light pale sepias known to be *EBCppF* by ancestry and breeding tests, but as low in intensity as 2 instead of the usual 8 to 13. In this case, it seemed best to exclude bodily a cluster of eight closely related matings in establishing the norm.

### *Correlations among residual variations*

It was obvious from the first that extreme dilution of yellow and of dark-eyed sepia were largely due to common factors. They tended to segregate out from the

[1] Paper No. 730 from the Department of Genetics, University of Wisconsin.

[2] This investigation was aided by a grant from the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago where the experimental work was conducted.

same matings, and, in tortoise-shells, in the same animals. Following are data on 233 tortoise-shells $e^{p}Bc^{d}c^{a}PFF$ from matings that could not produce any other $c$ genotype except the easily recognized intense black-red ($C$) or albino ($c^{a}c^{a}$). Most of them are from the experiments on extreme dilution since tortoise-shells were avoided in the main experiments.

| | 233 tortoise-shells | | 1949 norm | |
|---|---|---|---|---|
| | Av. | S.D. | Av. | S.D. |
| Sepia | 12.36 | 1.37 | 14.5 | 1.1 |
| Yellow | 3.05 | 0.78 | 4.2 | 0.6 |

$r = +.586 \pm .043$

It may be noted that the means of both sepia and yellow are far below those in the main experiment. The correlation would undoubtedly have been higher if a larger number of tortoise-shells of typical intensity for $c^{d}c^{a}$ had been included. It would also probably have been higher if more accurate grades had been used. In 29 of them in fact, galvanometer readings of intensity of both sepia and yellow were taken by means of a reflection meter. The correlation between suitable functions of these readings was $+.72 \pm .09$. There is a similar relation in browns.

The aberrantly light pale sepias ($pp$) came largely from unrelated matings. The pink-eyed sepias with gene $C$ that did segregate from matings that were producing exceptionally dilute yellows and dark-eyed sepias were of typical intensity (mean grade of 33 from such matings, $10.7 \pm .16$, in comparison with 9.7 from Table 1).

TABLE 1

*Average empirical grades and standard deviations of indicated genotypes as presented in a previous paper (Wright 1949)*

| | Black dark sepia $EBP(F,ff)$ | | Pale sepia $EBppFF$ | | Dark brown $EbbP(F,ff)$ | | Pale brown $EbbppFF$ | | Brownish cream $E(B,bb)ppff$ | | Stable yellow $ee(B,b;P,p)FF$ | | Fading yellow $ee(B,b;P,p)ff$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Av. | S.D. | Av. | S.D. | Av. | S.D. | Av. | S.D. | Av. | S.D. | Av. | S.D. | Av. | S.D. |
| $C-$ | 20.9 | 0.3 | 9.7 | 1.8 | 15.3 | 0.9 | 8.0 | 0.9 | 2.3 | 0.6 | 10.6 | 0.7 | 6.8 | 0.6 |
| $c^{k}c^{k}$ | 20.1 | 0.6 | 9.0 | 1.0 | 15.0 | 0.9 | 7.2 | 0.8 | 0.0 | 0.0 | 7.0 | 0.3 | 1.9 | 0.5 |
| $c^{k}c^{d}$ | 19.5 | 0.5 | 7.4 | 1.2 | 15.4 | 1.1 | 6.0 | 0.7 | 0.0 | 0.0 | 7.2 | 0.4 | 2.3 | 0.5 |
| $c^{k}c^{r}$ | 20.5 | 0.6 | 6.7 | 1.3 | 15.1 | 1.1 | 5.9 | 1.0 | .. | .. | 4.8 | 0.6 | .. | .. |
| $c^{k}c^{a}$ | 18.9 | 0.6 | 5.2 | 1.4 | 14.1 | 0.9 | 5.2 | 1.2 | 0.0 | 0.0 | 4.7 | 0.8 | 0.3 | 0.5 |
| $c^{d}c^{d}$ | 18.1 | 1.0 | 4.7 | 1.1 | 14.2 | 0.9 | 5.4 | 1.4 | 0.0 | 0.0 | 6.9 | 0.5 | 2.1 | 0.9 |
| $c^{d}c^{r}$ | 18.7 | 1.2 | 3.4 | 1.0 | 15.0 | 1.1 | 3.7 | 0.4 | .. | .. | 4.1 | 0.7 | 0.2 | 0.4 |
| $c^{d}c^{a}$ | 14.5 | 1.1 | 2.7 | 0.8 | 12.4 | 0.9 | 2.7 | 1.0 | 0.0 | 0.0 | 4.2 | 0.6 | 0.3 | 0.4 |
| $c^{r}c^{r}$ | 19.5 | 1.1 | 2.4 | 1.0 | 14.9 | 1.1 | 2.7 | 0.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| $c^{r}c^{a}$ | 15.0 | 1.7 | 0.7 | 0.6 | 12.5 | 1.1 | 1.0 | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| $c^{a}c^{a}$ | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

*Segregation of intensities of yellow with a given* c *genotype*

The grade of yellow of any genotype becomes uniform to the eye under close inbreeding. Any young from inbred strain No. 35 ($e^pe^pc^kc^kFF$) (WRIGHT 1922) could be used as the standard for grade 7 as long as this strain was on hand. Similarly, ones from strain No. 2 ($e^pe^pCCFF$) could be used as standard for grade 10 and ones from strain No. 32 (also $e^pe^pCCFF$ but with plus modifiers) were standard for grade 13.

In the color experiments, there were many matings between homozygous dilutes ($c^dc^d \times c^dc^d$, $c^kc^k \times c^kc^k$) with offspring that showed no apparent variation in the intensity of yellow *within* litters. In some of these all of the young of all litters were assigned grade 7, in others all were assigned grade 6, but often, some whole litters were assigned one grade and other litters another, e.g. grades 7 and 8 or grades 6 and 7. These progenies can not be interpreted as segregating. In grading litters, the grades assigned to previous litters were not looked up. As a unit difference is just perceptible to the eye by definition, litters from matings characterized by a grade between 7 and 8, and no appreciable variability, might be graded 7 in some cases, 8 in others merely by accident of subjective judgment, and similarly at lower levels.

The situation was similar in the case of matings that produced only a single heterozygous type, $c^kc^r$, $c^kc^a$, $c^dc^r$ or $c^dc^a$. There were many cases in which there was no recognizable variability within or among litters, all litters being of grade 5, all of grade 4 or all of grade 3, but also many cases in which some litters were graded 5, others 6, or some 4, others 5, or some 3, others 4.

Where there was segregation of *c* alleles, there was usually no doubt about the assignment of *c* genotypes because of the high correlation between the grades of the homozygotes and heterozygotes, especially within litters.

Many of the matings also produced sepias either as self sepias or in tortoiseshells to which both sepia and yellow grades were assigned. While the sepia grades in these cases showed less uniformity within litters than the yellows, they also showed increased variability between litters.

It was evident in inbred clusters of matings that these differences among the mean grades of progenies were hereditary. Since all of those referred to above are "dilute" in comparison with intense "red" or "black" it will be convenient to describe the differences considered here in terms of diminution: diminished as opposed to normal or undiminished. The grades of diminution referred to above will be referred to as moderate in contrast with extreme diminution taken up later.

In marked contrast to the types of progeny considered above were many progenies in which there was obvious segregation of grades of yellow of a given *c* genotype *within* litters. We find such records as (667) (6677) (666677) in three successive litters in which all young were $c^dc^d$, and (34) (334) (344) (3334) (4) (335) in six successive litters in which all young were $c^dc^a$. There was rarely any doubt as to whether a mating that produced yellows was of the

nonsegregating or segregating type. The situation was similar with respect to sepias but with more frequent ambiguity because of the variability within litters even where the yellow grades indicated homogeneity.

Consideration of clusters of matings which included apparent segregation of normality and moderate diminution indicated a unit difference in most cases. Moderate diminution often appeared to segregate out as if recessive. There were, however, cases in which higher grades segregated from lower within litters as if the lower grade were dominant. Making allowance for the subjective errors in the absolute grades of different litters, it is possible to interpret the results in most clusters on the basis of a pair of alleles with intermediate heterozygotes. On the other hand, there were some cases in which normal dilutes, breeding as such, traced to diminished ancestors in such a way as to indicate the presence of more than one locus with this effect.

Some of these same matings of moderately diminished parents produced diminution of a more extreme sort: yellows, $c^d c^d$, of grade 5 or even 4, and creams known to be $c^d c^a$, $c^d c^r$, $c^k c^a$ or $c^k c^r$ of grade 2 or 1. Many matings were made between such extreme types in the attempt to produce true breeding strains. These would be expected to be homozygous diminished if they were merely extreme variants of the moderately diminished type but they always produced strongly segregating progenies. In some cases, normals, breeding as such, were derived in two generations from ancestors that were all extreme.

### *Anomalous whites*

The clue to a fuller understanding of these variations in intensity came in the recognition that a hitherto unrecognized phenotypic class tended to appear whenever there was selection for extreme dilution. These were anomalous self whites or near-whites. They were overlooked for some time because of the possibility in many cases of interpretation in terms of known genes. Thus black-eyed whites might be interpreted as extreme piebald (*ss*) in progenies in which *ss* was segregating.

Self white as the extreme of spotting has, however, been uncommon in this stock except in an inbred strain (No. 13) characterized by the highest median percentage of white (97.8 percent) in its spotting pattern of any strain. It produced 24 percent self whites while strain No. 2 with a somewhat lower median, 93.8 percent, produced only three percent self whites (WRIGHT and CHASE 1936). Cases accumulated, moreover, in which this explanation was untenable because of the absence of spotting of any grade.

It also became clear that the eyes of the anomalous whites gave a dark red reflection more easily than those of black-eyed litter mates. This suggested that they might be red-eyed whites of genotype $eeBc^r c^r P$. This genotype was in fact produced by many matings of the extreme dilution experiments but suitable tests soon showed that $c^r$ was in fact absent from many other matings in which whites with dark red eyes were appearing. Neither of these explanations could account for cases in which such whites had small spots of very pale color on one or both

cheeks, overlooked in several of them until long after birth. A number of early breeding tests of these were frustrated by failure to produce any young.

In the early experiments on extreme dilution, there were 19 matings that produced whites or near-whites, 37 in number (in a total of 196 young) that could not be explained as of any hitherto known genotype. There were undoubtedly others that were producing this genotype but also producing red-eyed whites ($eec^rc^rP$), albinos, or extreme piebalds, from which it could not be distinguished with certainty. In seven of the above 19 matings, the anomalous near-whites could be distinguished but not the self whites. If one considers only the progenies in which the self whites as well as the near-whites must have been of this new genotype, there were 27 among 96 young. One of these produced 8 in 22, the others only one to three each. It appeared probable that, in some cases at least, those whites were segregating in a Mendelian 25 percent.

As noted in the preceding paper (WRIGHT 1959), what appeared to be the same type segregated from four matings of silvered black-red tortoise-shells from a cross of the pure silvered strain Ro with silvers from matings (LF) in which anomalous whites had appeared.

The new type was first definitely accepted as such in the progeny of a mating, L254, between unspotted pale yellows of grade 3 ($eec^dc^aPp$), not included above. This mating produced two dark-eyed near-whites and seven dark-eyed self whites among 22 dark-eyed young. There were also five pink-eyed yellows ($eec^dc^dpp$ and $eec^dc^app$) and nine pink-eyed whites, the latter largely, no doubt, $c^ac^a$, but possibly including anomalous pink-eyed whites. The self yellows included six of grade 5, two of grade 4, eight of grade 3, and one of grade 2, all below the typical grades for $c^dc^d$ and $c^dc^a$. One of the anomalous whites had a small pale yellow spot of grade 3 back of the right eye. Another appeared at birth to have a trace of very pale yellow (grade 1) on the head, which was verified later.

Descendants of this mating were inbred to the seventh generation (119 young). A much larger number (some 1300) were derived from a cross between it and a mating (L263), between a pale yellow of grade 2 from L254 and a sepia 14-yellow 4 tortoise-shell. The latter was considered to be typical of $e^peBBc^dc^aFFPP$ but probably was heterozygous for a diminution factor since many of the progeny were unexpectedly light. This mating produced no anomalous whites, however, and the same was true of four brother-sister matings made from it. Many appeared in later generations and also among some 2000 descendants of outcrosses.

### *Association of two kinds of diminution with anomalous whites*

Two different types of moderate diminution ultimately became fixed in different lines within this group. A clear case of one of these was in the line of inbreeding from L254 referred to above. A brother-sister mating (L282) between a yellow 5 ($c^dc^d$) and a yellow 3 ($c^dc^a$) produced only four young (two of grade 3 ($c^dc^a$) and two of grade 5 ($c^dc^d$)). The latter were mated (L298) to give a progeny consisting of seven self yellows of grade 6, 17 self yellows of grade 5, of

which at least four had such light bellies as to suggest silvering, and eight white or near-white. The six near-whites all had spots of much paler yellow (grade 3) than any of the self yellows, on one or both cheeks (two on right cheek only, two on left cheek only, and two on both). This progeny with exactly 75 percent self yellow of grades 5 or 6 and 25 percent self white or near-white with yellow head spots of grade 3 gives an unusually uncomplicated illustration of segregation of the new genotype from extreme diminished parents.

The gene for silvering (*si*) had not been suspected in the ancestry and neither parent in L298 had an especially light belly. It became clear in the next generation of brother-sister mating (W1) between a yellow 5 ($c^d c^d$) and a yellow 6 ($c^d c^d$), neither apparently silvered, that silver was indeed being transmitted. Two of the 14 self yellow young, (five of grade 5, eight of grade 6, one of grade 7), were strongly silvered on the back, and others had light bellies. In addition there were two near-whites (small head spot of grade 3 on each) and one self white. A brother-sister mating in the next generation (W8, yellow 5 × yellow 6, neither silvered) and two brother-sister matings from this (W30, both yellow 6; W33 both yellow 6, the dam strongly silvered) produced 23 yellow young which ranged from extreme diminished to fully typical in intensity (two (5), 16 (6), four (7), one (8)) of which four were considered to be strongly silvered and some half dozen others had bellies lighter than typical. None of these produced anomalous whites. A mating, W38, between two strongly silvered yellows of grade 6, one from W30, the other from W33, produced 15 yellow young (five (5), nine (6), one (7)) of which all but five of grade 6 were considered to be strongly silvered. Of these five, four were judged to have slightly light bellies, but one of grade 6 lacked even this indication of silvering. Breeding tests of one with a slightly light belly from this mating and a similar one from W33, by mating with strongly silvered black-reds from strain Ro, produced seven and five young respectively, all strongly silvered black-reds. The yellow parents thus were *sisi* in spite of the absence of actual silvering. None of the preceding four matings produced any anomalous whites among the 38 young. A brother-sister mating (W44) between two strongly silvered yellows of grade 5 from W38, on the other hand, produced an anomalous near-white (yellow 4 on nose) and two yellows of grade 6 with nearly white bellies and some irregular lightness on the back, like the others considered to be strongly silvered yellows. There is little doubt that matings W38 and W44 were both of type *sisi* × *sisi* and all of the others either *sisi* × *Sisi* or *Sisi* × *Sisi* but that the effect of *sisi* in these yellows (all $eec^d c^d$) was manifested more in diminution of intensity especially of the belly, than in actual silvering.

Diminution was not due wholly to effect of *sisi*, however. The same yellow 6 male as in W8 was mated (W32) with a yellow female, grade 5, whose father was also a yellow 6 from W1, mother a yellow 3 ($c^d c^a$) from the strain from L254 × L263). The 27 offspring, all yellow, consisted of 10 of grade 5, 15 of grade 6, and two of grade 7 of which only four were considered to have somewhat light bellies, but none so light as to indicate silvering. A male from the progeny (yel-

low 5) was mated back to the dam and produced eight young (one (4), three (5), three (6), one (7)), none silvered though two had somewhat light bellies. These two matings produced no anomalous whites.

Moderately diminished yellow not associated with silvering is shown more clearly in several lines derived from the crossbred group from L254 × L263. Thus one cluster of four matings of which three traced through one or two brother-sister matings to the first, included 84 young, all $c^dc^d$, of which 66 were of yellow grade 6 and eight of yellow grade 7 (ten with no yellow grade). None of these were recorded as having light bellies or any other distinctive indication of silvering. There were other clusters of matings that gave similar progenies. A cluster of six matings (YDL–1 and descendants) one called yellow 6 × yellow 7, the others all yellow 6 × yellow 6, produced one grade 5, 89 of grade 6, no anomalous whites and no distinctive suggestion of silvering in any case.

Further evidence for two kinds of diminution of color below the norm for the *c* genotype comes from breeding tests that have been made of 29 diminished with no overt silvering, by matings with strong silvers. Eight were clearly *SiSi*, 13 clearly *Sisi*, and eight probably *sisi*. Unfortunately, four of the latter are doubtful because of small number of offspring or the occurrence of overlaps. The best established were two yellows of grade 6, both $c^dc^d$, a pale yellow of grade 2 ($c^dc^a$) and a sepia 10-yellow 2 ($c^dc^a$). Of the doubtful ones, one was a yellow grade 5 ($c^dc^d$), two yellows of grade 2 ($c^dc^a$) and one sepia 10-yellow 2 ($c^dc^a$). Some of these were described as having very light bellies but none as clearly silvered. The grades of those shown to be *SiSi* or *Sisi* were rather similar ($c^dc^d$) sepia grades 15 to 18, $c^dc^a$ and $c^rc^a$ sepia grades 10 to 13, $c^dc^d$ yellow, all grade 6, $c^kc^r$, $c^kc^a$, $c^dc^r$, $c^dc^a$ all yellow of grade 3 except one grade 2 and one grade 4.

*The gene diminished* (dm): Seven matings were made between typical yellows (grade 7) (from a cluster that produced 34 of grade 7 and four of grade 8) and diminished yellows from the YDL–1 group above (grade 6). Three of these matings produced 29 young, all of grade 7, suggesting dominance of the higher grade, but the other four produced 11 of grade 6 in addition to 27 of grade 7. It is possible that the more intense parent was heterozygous, *Sisi*, in some of these cases. More accurate grades, based on reflection meter readings, indicate, however, that $F_1$ was really intermediate in all cases. The difference between visual grades 6 and 7 is too small for accurate judgments of dominance.

Nine backcrosses of $F_1$ yellow, grade 7, to diminished yellows grade 6 (of the same strain as used to produce $F_1$) gave 22 yellow-6 and 37 yellow-7 (no indication of silvering). The persistence of at least 50 percent of the $F_1$ type tends to indicate segregation at a single locus in these progeny which are descended 75 percent from the strain for which grade 6 is characteristic. We assume provisionally the existence of a gene *dm* (diminished) which reduces yellow of $c^dc^dFF$ from a strong grade 7 in *DmDm* to a weaker grade 7 in *Dmdm* and typically 6 in *dmdm*. Comparison of the sepia in clusters judged to be wholly *dmdm* by grade of yellow with that in typical $EBc^dc^dPDmDm$ indicates that the typical grades 18 or 19 are reduced to 16 or 17 in *dmdm*.

Matings in which there is segregation of yellows of *c* genotypes $c^dc^r$ or $c^dc^a$ indicate that "diminished" reduces the typical grade (a weak 5) to about grade 3. These do not show the light bellies often associated with similar diminution of color in these *c* genotypes by *sisi* (WRIGHT 1959). Similarly this type of dilution reduces sepia of *c* genotypes $c^dc^a$ from typical 15–16 to 12–13. In $c^rc^a$ the grades are about one unit higher.

The effect of *dmdm* differs markedly from that of *sisi* in not affecting intense black or yellow (*C*) which are usually strongly silvered by *sisi* and, at least in the latter, diluted. This may be illustrated by a mating (LC-29) between an unsilvered dilute yellow of grade 6, *eec*$^d$*c*$^d$*SiSidmdm* by ancestry, and an unsilvered intense yellow (grade 10), *eeCc*$^a$*SiSidmdm* also by ancestry. These produced 14 intense yellows of grade 10, three of grade 9 (the latter all in one litter) and 11 pale yellows all of grade 3 except for one called 2 and one called 4. There was no appreciable diminution of the intense yellows (*Cc*$^d$*dmdm*). They were indeed unusually intense on the average by the reflection meter readings. The marked reduction from the typical intensity of *c*$^d$*c*$^a$*dmdm* yellows was accompanied in only one case by a slightly greater than usual reduction on the belly.

### *The parents of anomalous whites*

The characteristics of the parents of the anomalous whites must be taken into account in drawing conclusions on their genetics. Tables 2 and 3 show by *c* genotypes the grades of sepia and yellow respectively of all mated individuals that have produced this type and have grades. Tortoise-shells are entered under both their sepia and yellow grades. The average grades in the colony are repeated from Table 1 for comparison. As already noted, it is now apparent that some of these averages, especially those for genotypes involving $c^d$, are too low to be typical for genotypes that lack diminution factors since it has become clear that one of these

TABLE 2

*Sepia (or brown) grades of parents of anomalous whites. No grades were assigned to silvered black in seven cases (six tortoise-shells) and a silvered red-eyed dark sepia* ($c^rc^r$ sisi). *Two pale sepias* (E, $e^p$ B $c^dc^a$ pp) *of grades 2 and 3 (1949 norm 2.7) are not entered*

| | AW | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | S? | Total (Excl. AW) | Mean | 1949 Norm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C* | . | . | . | . | . | . | . | . | . | . | . | 3 | . | . | 3 | 20.0 | 20.9 |
| *C sisi* | 2 | . | . | . | . | . | . | . | . | . | . | . | 3 | (7) | 10 | 21.0 | ... |
| $c^kc^a$ | . | . | . | . | . | . | . | . | 1 | 1 | . | . | . | . | 2 | 17.5 | 18.9 |
| $c^dc^d$ | . | . | . | . | . | 1 | . | . | . | . | . | . | . | . | 1 | 14.0 | 18.1 |
| $c^dc^r$ | . | . | . | . | . | . | 1 | 1 | 1 | . | 1 | . | . | . | 4 | 16.7 | 18.7 |
| $c^dc^a$ | 2 | 4 | 9 | 2 | 1 | . | . | . | . | . | . | . | . | . | 16 | 11.0 | 14.5 |
| $c^rc^r$ *sisi* | . | . | . | . | . | . | . | . | . | . | . | . | . | (1) | 1 | ... | ... |
| $c^rc^a$ | . | 1 | 3 | 2 | . | . | . | . | . | . | . | . | . | . | 6 | 11.2 | 15.0 |
| $c^dc^a$ *bb* | . | 2 | . | . | . | . | . | . | . | . | . | . | . | . | 2 | 10.0 | 12.4 |

TABLE 3

*Yellow grades of parents of anomalous whites. No grades were assigned to yellow in seven intense silvered tortoise-shells*

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Y? | Total | Mean | 1949 Norm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C* | . | . | . | . | . | . | . | . | 1 | 1 | . | 2 | 9.5 | 10.6 |
| *C sisi* | . | . | . | . | . | . | 1 | 5 | 1 | 1 | (7) | 15 | 8.2 | . . . |
| *C ff* | . | . | . | . | . | . | 2 | . | . | . | . | 2 | 7.0 | 6.8 |
| $c^k c^k$ | . | . | . | . | . | 1 | . | . | . | . | . | 1 | 6.0 | 7.0 |
| $c^k c^r$ | . | . | 1 | . | . | . | . | . | . | . | . | 1 | 3.0 | 4.8 |
| $c^k c^a$ | . | 1 | 3 | 1 | . | . | . | . | . | . | . | 5 | 3.0 | 4.7 |
| $c^d c^d$ | . | . | . | 1 | 5 | 1 | . | . | . | . | . | 7 | 5.0 | 6.9 |
| $c^d c^d$ *sisi* | . | . | 1 | 1 | 3 | 2 | . | . | . | . | . | 7 | 4.9 | . . |
| $c^d c^r$ | . | 4 | 9 | . | . | . | . | . | . | . | . | 13 | 2.7 | 4.1 |
| $c^d c^a$ | . | 12 | 20 | 2 | . | . | . | . | . | . | . | 34 | 2.7 | 4.2 |

(*dm*) was much more frequent in this main series of color experiments than had been suspected.

The data point in two directions. With respect to intense black parents (*BCP*), it may be noted that ten showed overt silvering and only three did not, apart from two that were anomalous whites themselves. Of the three unsilvered, two were known to be heterozygous, *Sisi*, because of a silvered parent or offspring and the other came from a mating of type *Sisi* × *Sisi*. With respect to intense yellow parents (*CFF*), 15 were overt silver and only two not, but both of these came from *Sisi* × *Sisi*. Allowing for eight tortoise-shells, there were 24 *C*-parents, of which 17 were silvers (*sisi*), two definitely *Sisi*, and three may well have been *Sisi* as coming from *Sisi* × *Sisi*, and two were anomalous whites.

The suggestion that anomalous whites are modified silvers, *sisi*, is unavoidable, as silvering was not common in the colony outside of strain Ro and derivatives, which were the source of only six of the above 17 silver parents.

Among parents, with lower *c* alleles, however, relatively few were overt silvers. There was one $c^r c^r$ silvered black from *sisi* × *sisi* among 30 with sepia grades, and seven $c^d c^d$ silvered yellows among 68 with yellow grades, or eight silvers among 80 parents, allowing for 18 tortoise-shells. In addition, six $c^d c^d$, three $c^d c^r$, seven $c^d c^a$ and one $c^r c^a$ are known by a silver parent or offspring to carry *si*. Thus the parents (L350) of two anomalous whites, which at the time were not known to have silver ancestry, were tested by mating with silvered black-reds from strain Ro. The male, a pale yellow of grade 2 ($c^d c^a$) turned out to be *sisi*, and the female, sepia 11-cream 3 ($c^d c^a$), turned out to be *Sisi*. The 55 in which presence or absence of *si* was unknown from appearance of *sisi* in a parent or offspring, were all closely related to silvers and there were none which can be asserted to be *SiSi*. There were also two parents, with lower *c* alleles, that were anomalous whites themselves.

On examining the sepia and yellow grades of the dilute parents of anomalous whites it may be seen that all of the yellow grades (except perhaps two $c^d c^a$ of

yellow grade 4) and all of the sepia grades of $c^d c^a$ and $c^r c^a$ are below, and most of them far below the 1949 averages for their genotypes. To some extent this merely reflects the fact that anomalous whites have appeared only in experiment on extreme dilution (apart from ten from RoLF) but this is not the full explanation since many of the matings in these experiments involved typical grades. There is, in short, a strong implication that genes making for extreme dilution must be received from both parents to produce anomalous whites.

The complete absence of overt silvering among the many $c^d c^a$ parents is somewhat disconcerting. It has, however, been brought out earlier and in the preceding paper (WRIGHT 1959) that overt silvering is almost completely suppressed both in sepias and yellows of this genotype unless there is an accumulation of modifiers for very strong silvering as in derivatives of strain Ro. There is instead a uniform, moderate diminution of color on the back and a more extreme diminution on the belly.

There were two dilute browns, ($e^p bbc^d c^a P$), among the parents of anomalous whites, both of grade 10, which is less than the 1949 average, 12.4. There were two fading yellows (*eeCff* of grade 7) which gave no indication of any diminution of color below the norm, 6.8. There were two pink-eyed pale sepias $Ee^p Bc^d c^a$ of grades 2 and 3 which also gave no indication of excess diminution of color (1949 norm 2.7). Finally there were 22 albinos ($c^a c^a$) and four red-eyed whites ($eeBc^r c^r P$). One of the albinos was certainly and two probably *Sisi* by test. All of the animals above were related to ones known to transmit *si* and none were known to be *SiSi*.

### *Anomalous white as diminished silver* (sisidmdm)

A considerable number of anomalous whites were mated with silvers but none of the males produced any young and the females few. One such female (from L298), however, produced 11 offspring, all silvered. Another produced four and two others three each, all silvered. One of them, in a mating with a brother ($c^d c^a$, pale yellow, grade 2), produced two anomalous whites, and no other classifiable young. As far as they go, these direct tests confirm the view that anomalous whites are *sisi*.

Their appearance is more in harmony with this interpretation than was realized until outcrosses and selection of silvered black-reds (strain RoXR, WRIGHT 1959) produced extreme silvers which bridged the phenotypic gap between the previously observed strong silvers with mere sprinkling of white over the back, nearly white belly, unsilvered head and feet, and the near-whites and whites from the experiments on extreme dilution. The three most extreme silvers in RoXR (grade 12) were like the anomalous near-whites in restriction of color to spots on the head and diminished intensity and silvering of these spots. The anomalous whites may be considered as silvers in which blotching has reduced color to small spots on the head (and rarely a few colored hairs on the back) and in which silvering and diminution are extended to these head spots. The anomalous white males differ from the extreme silvered males of RoXR in being sterile.

While it seems clear the anomalous whites are *sisi*, the modifier or modifiers that make them extreme seem to have no relation to those in RoXR. The diminution of intensity of color of their parents, below that expected from their *c* genotypes can be due only in part to the effect of *sisi* since it occurs where this parent is *Sisi* and exceeds that due to *sisi* alone when this parent is *sisi*. The extraction of another type of diminution (*dmdm*), not associated with a silvering effect, from the lines that produce anomalous whites suggests that the genotype of the latter is *sisidmdm*.

### *Analysis of a large network of matings*

A systematic attempt was made to test whether the data from the large, rather tightly knit group descended from L254 and L263, and outcrosses is consistent with this interpretation. After considerable experimentation the following rough norms were set up for the effects of *si* and *dm* in sepia and yellow in the *c* genotypes of most importance in the above group of matings (Table 4).

In this scheme, the same diminution effect is attributed to *si* and *dm*. This must be qualified in the case of *eeC*- in which silvering (*sisi*++) has seemed to be clearly associated with reduction from yellow of grade 10 or more to about grade 9 while genotype ++*dmdm* is apparently not associated with any diminution. These grades refer to the dorsal color. Ventrally, *si* seems to be associated with a greater reduction of intensity than *dm*. Finally *si* tends to be associated with sprinkling with white hairs in the higher *c* genotypes (*C* and usually $c^dc^d$) while no effect of this sort whatever can be attributed to *dm*. Indeed there is no evidence that *dm* behaves as a modifier that extends silvering in the narrow sense except in *sisidmdm*.

It is not likely that the effects of *si* and *dm* are really identical for lower *c* alleles. An initial attempt, however, to interpret *si* as having a more extreme diminution effect than *dm* throughout, based on particular cases, broke down. An attempt to interpret +*si*+*dm* as more intense than *sisi*++ or ++*dmdm* (i.e.

TABLE 4

*Postulated typical grades of* si -dm *genotypes in sepias and yellows in most frequent* c- *genotypes*

| | Sepia | | | | Yellow | | |
|---|---|---|---|---|---|---|---|
| | *E-C-* | *E-$d^dc^d$* | *E-$c^dc^a$* | *E-$c^rc^a$* | *eeC-* | *ee$c^dc^d$* | *ee($c^dc^a$,$c^dc^r$)* |
| ++++ | 21 | 18–19 | 15–16 | 16–17 | 10 | 8 | 5 |
| +++*dm*<br>+*si*++ | 21 | 17–18 | 14–15 | 15–16 | 10 | 7 | 4 |
| ++*dmdm*<br>+*si*+*dm*<br>*sisi*++ | 21 | 16–17 | 12–13 | 13–14 | 10<br>(9) | 6 | 3 |
| +*sidmdm*<br>*sisi*+*dm* | 20 | 14–15 | 10–11 | 11–12 | 9 | 5 | 2 |
| *sisi dmdm* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

to interpret *si* and *dm* as more nearly recessive than dominant) also broke down. The evidence on *sisi*++ from the above group of matings is not very extensive. There is some reason from other matings referred to in the preceding paper (WRIGHT 1959) to consider that this actually has slightly less diminution effect than ++*dmdm*. The hypothesis of equal diminution effects here, however, seemed to be an adequate first approximation for analysis of these data.

There were indications that the effects on $c^k$ genotypes are similar but slightly less than on $c^d$ genotypes. There were also indications of effects on brown, parallel to those on sepia (little or no diminution with *C*, appreciably more with lower *c* alleles). In the pale sepias, there was no evidence of any effect in the presence of *C* except for silvering of *sisi*. There were probably effects in the presence of lower *c* alleles, but the evidence was very scanty.

The attempt was made to assign a formula to every mating in this group of matings on the basis of (1) the possibilities provided by the formulae of the parental matings, (2) the grades of the mated individuals, (3) the general level of intensity of the progeny, (4) the indications of segregation in intensity in the progeny, (5) the occurrence and frequency of anomalous whites in the progeny and finally (6), the results of all breeding tests of offspring. Especial weight was given in (2), (3), and (4) to grades obtained by reflection meter readings of some 1,500 young in these experiments which are not discussed in this paper. In the end, as a result of (1) and (6), the formulae throughout the entire network of matings form a self-consistent set.

No doubt errors have been made in these assignments, especially in matings with small number of offspring and few or no breeding tests of the latter. Such cases are relatively unimportant. There was usually no choice in the case of the key matings with many offspring and several of these mated. Analysis of the reflection meter grades will be made elsewhere.

The important point here is that it proved possible at all to obtain a consistent interpretation of this entire network of matings without doing serious violence to the observed grades at any point.

### *Segregation of anomalous whites*

The only cases in which there is sufficient phenotypic discontinuity to permit clear-cut Mendelian ratios are those in which *sisidmdm* segregates from other genotypes. There are nine possible types of mating with respect to *si* and *dm* from which such segregation is expected (combining reciprocal types and assuming that *sisidmdm* males are sterile, a matter discussed in detail later).

There were 59 matings in the cluster in which genotypes were assigned, that were capable of producing *sisidmdm*. These include 17 which did not have any offspring that were certainly of this genotype. The offspring of each mating, excluding one discussed later, were classified into the following categories, using $c^x$ for a *c* allele other than $c^a$.

(1) Dark eyed ($c^x$-*P*-) from matings incapable of producing $eec^rc^{ra}P$.
(2) Pink eyed ($c^x$-*pp*) from matings incapable of producing $c^ac^a$.

(3) Pink-eyed white ($c^ac^aP$-) from matings incapable of producing *pp*.
(4) Pink eyed from matings capable of producing both $c^ac^a$ and *pp*.

Those from (1), (2) and (4) that are neither white nor near-white are considered as not *sisidmdm*. In categories (1) and (2), whites and near-whites are considered to be *sisidmdm*. There is a possibility of confusion from high grade spotting due to *ss* which segregated out in a few matings. It is believed that errors of classification of near-whites were avoided by taking account of size and intensity of the head spots. It is not likely that there were any errors from the rare occurrence of self white due to *ss*. Category (3) cannot be classified as *sisidmdm* or not, by appearance. One albino male (mating type 8) was, however, undoubtedly $c^ac^a$*sisidmdm* (very small testes). Another (mating type 5) was probably of this genotype (sterile in seven months of mating after maturity). In category 4, pink-eyed near-whites are definitely $c^r$-*ppsisidmdm* but pink-eyed self whites of this genotype cannot be distinguished by appearance from albinos ($c^ac^a$) which may or may not be $c^ac^a$*sisidmdm*. One self white male was undoubtedly *sisidmdm* (very small testes) and two others (mating types 3 and 8 were sterile in matings of three and six months duration respectively. The expected numbers of $c^r$-*ppsisidmdm* and of albinos were calculated for each mating but no attempt was made to apportion the former between near-white and white.

Genotypes $c^rc^r$ and $c^rc^a$ segregated from several matings but there was only one (L358, mating type 5) in which *ee* also segregated out making possible red-eyed white of genotype $eec^rc^aPP$ which might be mistaken for anomalous white. This mating ($e^be^dc^rPP \times e^be^ac^aP$-) produced 13 definitely not near-white or white, two definitely near-white (*sisidmdm*) and seven dark eyed whites (four born dead) which cannot be classified with respect to *sisidmdm*. This mating is excluded from Tables 5 and 6.

Table 5 gives a summary of the offspring of the 58 matings, according to mating type and the above phenotypic and genotypic categories. There are no important deviations from expectation, if the near-whites and self whites of columns 9 and 10 are combined. ($\chi^2=17.7$, n=26 on grouping entries that are unduly small) From the grand totals it appears that only about 23 percent ($=\frac{4}{16.9}$) of $c^r$-*ppsisidmdm* (column 9 expected) are near-white (column 9 observed) in progenies in which those that are self white are indistinguishable from albinos and thus are included in column 10.

Table 6 gives a summary by expected ratio, restricted to the categories 1 and 2 combined, in which all whites and near-whites can be attributed with confidence to *sisidmdm*. Agreement is good ($\chi^2=2.8$, n=4).

This agreement is favored to some extent by circular reasoning since ratio was taken into account in assigning formulae. It was, however, only one of the six things that were taken into account, as has been noted. The important point is that the data can be interpreted consistently with the theory in this matter as well as in all others.

TABLE 5

*Offspring of matings derived from L254, L263 and outcrosses that were capable of producing anomalous white (AW=*sisidmdm*). These matings are classified by type with respect to* si *and* dm*. Offspring are classified as dark eyed or pink eyed. Pink eyed* ($c^x$-pp) *from matings incapable of producing albinos, and dark eyed are unequivocally AW or not AW. Albinos* ($c^ac^a$P) *from matings incapable of producing* pp *cannot be classified phenotypically with respect to AW. Pink eyed from matings capable of producing both* $c^ac^a$ *and* pp *can be classified as AW if near-white, as not AW if neither white nor near-white but are unclassifiable if self white. Expected values are given for all classes except those for column 9 include all* $c^x$-ppsisidmdm, *near white or self white while that for column 10 is for* $c^ac^a$ *and thus does not include self white* $c^x$-ppsisidmdm *which is included in the observed figure*

| | Mating type | No. matings | Dark eyed ($c^x$-P-) AW | Dark eyed ($c^x$-P-) Not AW | Pink eyed ($c^x$-pp) AW | Pink eyed ($c^x$-pp) Not AW | Pink eyed ($c^ac^a$P) Self W (?) | Pink eyed ($c^x$-pp, Near-W AW | Pink eyed $c^ac^a$P or $c^ac^a$pp Self W (?) | Pink eyed $c^ac^a$pp Not W Not AW | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *sisi* + *dm* × *sisi dmdm* | 2 | 3 | 7 | .. | .. | .. | 0 | 1 | 1 | 12 |
| | | | 4.5 | 4.5 | .. | .. | .. | (0.6) | (1.7) | 0.7 | |
| 2 | + *si dmdm* × *sisi dmdm* | 1 | 4 | 4 | .. | .. | .. | .. | .. | .. | 8 |
| | | | 4.0 | 4.0 | .. | .. | .. | .. | .. | .. | |
| 3 | *sisi* + *dm* × *sisi* + *dm* | 5 | 5 | 18 | 2 | 5 | 6 | 0 | 6 | 6 | 48 |
| | | | 6.3 | 18.8 | 1.7 | 5.3 | 4.5 | (1.2) | (6.5) | 3.7 | |
| 4 | *sisi* + *dm* × + *si dmdm* | 11 | 30 | 77 | 18 | 59 | 2 | 1 | 39 | 16 | 242 |
| | | | 26.0 | 78.1 | 19.3 | 57.8 | 1.7 | (6.4) | (33.5) | 19.2 | |
| 5 | + *si dmdm* × + *si dmdm* | 15 | 22 | 67 | 1 | 0 | 26 | 2 | 55 | 13 | 186 |
| | | | 22.1 | 66.2 | 0.5 | 1.5 | 31.5 | (6.2) | (39.5) | 18.5 | |
| 6 | + *si* + *dm* × *sisi dmdm* | 0 | .. | .. | .. | .. | .. | .. | .. | .. | 0 |
| 7 | + *si* + *dm* × *sisi* + *dm* | 2 | 2 | 17 | .. | .. | 6 | .. | .. | .. | 25 |
| | | | 2.0 | 13.8 | .. | .. | 9.2 | .. | .. | .. | |
| 8 | + *si* + *dm* × + *si dmdm* | 14 | 14 | 80 | 0 | 8 | 58 | 1 | 27 | 16 | 204 |
| | | | 12.1 | 84.5 | 1.0 | 7.0 | 54.5 | (2.5) | (25.0) | 17.4 | |
| 9 | + *si* + *dm* × + *si* + *dm* | 8 | 1 | 55 | 1 | 19 | .. | .. | .. | .. | 76 |
| | | | 3.7 | 56.0 | 1.0 | 15.3 | .. | .. | .. | .. | |
| | Total | 58 | 81 | 325 | 22 | 91 | 98 | 4 | 128 | 52 | 801 |
| | | | 80.7 | 325.9 | 23.5 | 86.9 | 101.4 | (16.9) | (106.2) | 59.5 | |

TABLE 6

*Summary of data in columns 4 to 7, Table 5, combining young from categories in which classification as definitely* sisi dmdm *or not is possible. Expected values based on totals for these categories*

| Mating type | Expected Not AW:AW | Not *sisi dmdm* Obs. | Not *sisi dmdm* Exp. | *sisi dmdm* Obs. | *sisi dmdm* Exp. | Total |
|---|---|---|---|---|---|---|
| (1) (2) | 1:1 | 11 | 9.0 | 7 | 9.0 | 18 |
| (3) (4) (5) | 3:1 | 226 | 228.0 | 78 | 76.0 | 304 |
| (7) (8) | 7:1 | 105 | 105.9 | 16 | 15.1 | 121 |
| (9) | 15:1 | 74 | 71.2 | 2 | 4.8 | 76 |
| Total | | 416 | 414.1 | 103 | 104.9 | 519 |

## *The colored spots of guinea pigs of genotype* sisidmdm *(silver-whites)*

We begin here a more detailed consideration of the characteristics of genotype *sisidmdm*, which it will now be convenient to call "silver-white." Tables 7 and 8

TABLE 7

*Numbers of dark-eyed silver-whites called white at birth but spotted later* W', *of others white at birth* (W), *and numbers and grades of sepia and yellow spots at birth. R indicates red-eyed segregant,* $c^rc^a$. *A from matings from which self whites must be* sisidmdm. *B from matings in which self whites need not be* sisidmdm *and are not entered. One with a tortoise-shell spot is indicated by an asterisk*

| | White W | White W' | Sepia No. | Sepia Grade | Yellow No. | Yellow Grade | Total A | Total B |
|---|---|---|---|---|---|---|---|---|
| *C* | . | . | 1 | (17) | 3 | (7,7,8) | 3 | 1 |
| *Cff* | . | . | . | ..... | 1 | (4) | . | 1 |
| $c^kc^a$ | 1 | . | . | ..... | 1 | (2) | 2 | . |
| $c^dc^a$ | 25 | . | 4 | (4,5,7,10) | 1 | (1) | 29 | 1 |
| $c^rc^a$ | 1 | . | 1 | (7) | . | ..... | 1 | 1 |
| $1C{:}1c^kc^a$ | 1 | . | . | ..... | 1 | (8) | 2 | . |
| $1C{:}1c^dc^d$ | . | . | 2 | (13,15) | . | ..... | 2 | . |
| $2C{:}1c^dc^d{:}1c^dc^a$ | 3 | . | 1 | (19) | . | ..... | 4 | . |
| $2C{:}1c^dc^r{:}1c^dc^a$ | 1 | 1 | 2 | (5,11) | . | ..... | 4 | . |
| $2C{:}1c^dc^a$ | 2 | . | 3 | (8,14,15*) | 2 | (6,7*) | 4 | 2 |
| $1C{:}1c^dc^a$ | 1 | . | 2 | (14,14) | 1 | (3) | 4 | . |
| $1c^kc^k{:}1c^kc^a$ | 1 | . | . | ..... | . | ..... | 1 | . |
| $1c^kc^k{:}2c^kc^a$ | 1 | . | . | ..... | 3 | (1,1,3) | 4 | . |
| $1c^kc^r{:}1c^rc^r$ | . | 1 | . | ..... | . | ..... | . | 1 |
| $1c^dc^d{:}1c^dc^r{:}1c^dc^a{:}1c^rc^a$ | . | . | 2 | (4R,6) | . | ..... | . | 2 |
| $1c^dc^d{:}1c^dc^a$ | 3 | . | . | ..... | 1 | (3) | 4 | . |
| $1c^dc^d{:}2c^dc^a$ | 15 | 3 | 1 | (8) | 3 | (2,3,4) | 22 | . |
| $1c^rc^r{:}2c^rc^a$ | 2 | . | 8 | (2,2,3,7, 7,8,8,11) | . | ..... | 10 | . |
| A | 57 | 4 | 21 | ..... | 14 | ..... | 96 | . |
| B | .. | 1 | 6* | ..... | 3* | ..... | . | 9 |

TABLE 8

*Numbers and grades of pink-eyed silver-whites. Otherwise as in Table 13. One with a tortoise-shell spot is indicated by an asterisk*

| | White | | Sepia | | Yellow | | Total | |
|---|---|---|---|---|---|---|---|---|
| | W | W′ | No. | Grade | No. | Grade | A | B |
| $c^k c^r$ | . | . | . | ..... | 1 | (2) | . | 1 |
| $c^d c^d$ | 3 | . | . | ..... | 8 | (3,3,3,3, 3,3,3,4) | 11 | . |
| $c^d c^r$ | 10 | 1 | . | ..... | 2 | (1,1) | 13 | . |
| $c^d c^a$ | . | 1 | . | ..... | 1 | (2) | . | 2 |
| $1C{:}1c^k c^a$ | 1 | . | . | ..... | . | ..... | 1 | . |
| $1C{:}1c^d c^d$ | 2 | 1 | . | ..... | 1 | (6) | 4 | . |
| $2C{:}1c^d c^d{:}1c^d c^a$ | 1 | . | . | ..... | . | ..... | 1 | . |
| $2C{:}1c^d c^r{:}1c^d c^a$ | 2 | 1 | . | ..... | 1 | (8) | 4 | . |
| $1c^k c^k{:}2c^k c^a$ | . | . | . | ..... | 1 | (3) | . | 1 |
| $1c^d c^d{:}1c^d c^r$ | 1 | . | . | ..... | 1 | (1) | 2 | . |
| $1c^d c^d{:}2c^d c^r$ | . | . | . | ..... | 5 | (1,2,2,2,2,) | . | 5 |
| $1c^d c^d{:}2c^d c^a$ | . | . | . | ..... | 2 | (2,2) | . | 2 |
| $1c^d c^d{:}2c^d c^{ra}$ | . | . | 1 | (2*) | 1 | (2*) | . | 1 |
| $1c^r c^r{:}2c^r c^a$ | . | 1 | . | ..... | . | ..... | . | 1 |
| A | 20 | 3 | . | ..... | 13 | ..... | 36 | . |
| B | . | 2 | 1* | ..... | 11* | ..... | . | 13 |

give a classification by possible *c* genotype and grade of all recognized silver-whites (dark-eyed (*P*) in Table 7, pink-eyed (*pp*) in Table 8). Those that were self-white as far as observed are called W. These are entered only from matings (A) that could produce no other genotype that could be confused with *sisidmdm*. Those that were recorded as white at birth but on which very pale cream (in two cases with a few sepia hairs) was observed later on the cheeks, are called W′ and are entered both for matings of type A and also from the other matings (B). Many of those called W were born dead or died soon after birth.

In many cases *c* genotypes can be definitely assigned from the nature of the mating. In a larger number, however, there was segregation of the two or more *c* genotypes, and the genotype can only be inferred from the intensity of the head spots if any. The self-whites can only be apportioned on the basis of expected ratios.

We note first that the head spots of known *C*'s may be of sepia grade 17 and yellow of grade 7 or 8. It may also be seen that several yellows of grades 6 to 8 are found where *C* is expected (both among dark-eyed and pink-eyed young), but none above grade 4 otherwise. The highest grade of sepia assigned any possible *C* segregant is 19. A tortoise-shell, sepia 15-yellow 7, indicates that the grade of *C-sisidmdm* may be at least as low as 15. Grade 14 occurs where $c^d c^a$ is the only other *c* genotype than *C*, and in view of other data may be assigned the latter. We conclude that sepia head spots of *C sisidmdm* range from grade 14 to 19. The distributions for this and other genotypes are shown in Table 9. The average for seven believed to be *C* is $15.4 \pm 0.7$. Similarly, we conclude that yellow head spots of *C-sisidmdm* range from grade 6 to 8 (irrespective of *P* or *pp*). The dis-

TABLE 9

*Grades of sepia at birth in colored spots of* sisidmdm. *The* c *genotypes apportioned in segregating litters as described in the text*

| | Intensity of sepia spots | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | Total | Mean |
| *C* | . | . | . | . | . | . | . | . | . | . | . | . | . | 3 | 2 | . | 1 | . | 1 | 7 | 15.4±0.7 |
| $c^dc^d$ | . | . | . | . | . | . | . | . | . | . | . | . | 1 | . | . | . | . | . | . | 2 | 12.0±1.0 |
| $c^dc^r$ | . | . | . | . | . | . | . | . | . | . | 1 | . | . | . | . | . | . | . | . | | |
| $c^dc^a$ | . | . | . | 1 | 2 | 1 | 1 | 2 | . | 1 | . | . | . | . | . | . | . | . | . | 8 | 6.6±0.7 |
| $c^rc^r$ | . | . | . | . | . | . | . | 2 | . | . | 1 | . | . | . | . | . | . | . | . | 3 | 9.0±1.0 |
| $c^rc^a$ | . | 2 | 1 | 1 | . | . | 3 | . | . | . | . | . | . | . | . | . | . | . | . | 7 | 4.6±0.9 |

tribution for yellows is given in Table 10. The average for eight believed to be *C* is 7.12 ± .30. These averages are not only much lower than expected of typical *C* (black 21, yellow 10 or 11) but also lower than in genotype *C-sisiDmdm* (black about 20, yellow about 9).

It should be added that in all of these cases the head spots were strongly silvered with white hairs. The grades are intended, however, to apply to the unsilvered hairs. In one case, a few scattered sepia hairs were observed on the back of an otherwise dark-eyed white.

Yellow in known $c^dc^d$*sisidmdm* was of grade 3 in seven cases and grade 4 in one. It was probably as low as grade 2 in segregating progenies. What little evidence there is on $c^kc^k$*sisidmdm* indicates grade 3 as typical for this also. The distributions are shown in Table 10 based, in segregating progenies, on the expected ratios and the assumption that $c^dc^d$ and $c^kc^k$ tend to be more intense than $c^kc^r$, $c^kc^a$, $c^dc^r$, and $c^dc^a$. The mean for $c^kc^k$*sisidmdm* and $c^dc^d$*sisidmdm* combined is 3.00 ± .14. This again is not only much lower than for typical $c^kc^k$ or $c^dc^d$ (about 7) but also for $c^dc^d$*Sisidmdm* or $c^dc^d$*sisiDmdm* (about grade 5). The head spots in these cases appeared to be uniform pale yellow rather than silvered.

Genotypes $c^kc^r$, $c^kc^a$, $c^dc^r$, and $c^dc^a$ typically have yellow of about grade 5. This is reduced to about grade 2 as a rule when the above *c* genotypes are combined with *Sisidmdm* or *sisiDmdm*. The head spots of near-whites, *sisidmdm* of these *c* genotypes are assigned grades 1 or 2 with one dubious exception (grade 3 in an animal born dead and wet when found). The average for the 17 believed to be of

TABLE 10

*Grades of yellow at birth in colored spots of* sisidmdm. *The* c *genotypes apportioned in segregating litters as described in the text*

| | Intensity of yellow spots | | | | | | | | Total | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | |
| *C* | . | . | . | . | . | 2 | 3 | 3 | 8 | 7.12±.30 |
| $c^kc^k$ | . | . | 2 | . | . | . | . | . | 15 | 3.00±.14 |
| $c^dc^d$ | . | 2 | 9 | 2 | . | . | . | . | | |
| $c^kc^r$ | . | 1 | . | . | . | . | . | . | 17 | 1.65±.15 |
| $c^kc^a$ | 2 | 1 | . | . | . | . | . | . | | |
| $c^dc^r$ | 3 | . | . | . | . | . | . | . | | |
| $c^dc^a$ | 2 | 7 | 1 | . | . | . | . | . | | |

these genotypes was 1.65 ± .15. This, however, is undoubtedly too high since the great majority in these cases were recorded as self white at birth. We have not indeed included the eight probably of these genotypes which were recorded as self white at birth but in which very pale cream spots were observed later.

The grades of sepia in head spots of four known *c$^d$c$^a$sisidmdm* indicate a range of at least 4 to 10. Apportionment in segregating litters suggests four more cases, in this range, with average for all eight of 6.6 ± 0.7. In the case of *c$^r$c$^a$sisidmdm* there are two known cases of grades 4 and 7. There are probably five other cases, ranging from 2 to 7 from three matings segregating in *c$^r$c$^a$* and *c$^r$c$^r$*. The average for these seven is 4.6 ± 0.9. These were silvered but this could be because they were tortoise-shells. These averages are again far below the typical grades of *c$^d$c$^a$* and *c$^r$c$^a$* (15 to 17) and below those for *c$^d$c$^a$* or *c$^r$c$^a$* combined with *Sisidmdm* or *sisiDmdm* (about 10 to 12).

With the apportionment indicated above for progenies segregating in *c$^r$c$^r$* and *c$^r$c$^a$*, three cases of *c$^r$c$^r$sisidmdm* range from sepia grades 8 to 11 with average 9.0 ± 1.0, far below the typical grade of about 19 or 20. The average for *c$^r$c$^r$Sisi dmdm* and *c$^r$c$^r$sisiDmdm* are uncertain but is probably about 17 or 18. One of those considered *c$^r$c$^r$sisidmdm* (grade 8) was one of three silver-whites observed to have a few dark hairs on the back.

There are few data on the intensity of sepia in head spots of other *c* genotypes. One of grade 13 is probably *c$^d$c$^d$* and one of grade 11 is probably *c$^d$c$^r$*, which as in the other cases are far below the typical grades.

There was only one pink-eyed silver-white with pale sepia in a head spot. This was a pink-eyed white with a pale sepia 2-yellow 2 spot, probably *c$^d$c$^d$ppsisi dmdm*. The typical grade for *c$^d$c$^d$pp* is about 4.7. It is notable that dilute pale sepia is not completely eliminated.

There was one probable fading yellow of genotype *eeC-ffsisidmdm*. This was a near-white with a head spot of grade 4 that segregated out from a brother-sister mating (LF122), both parents probably *eeCc$^r$ffSisiDmdm*, that produced 13 self or near-self yellows of grades 6 to 8 (*eeCff*), seven red-eyed whites (*eec$^r$c$^r$ff*) and the above near-white. It is remarkable that no silvered yellows (*eeC-ffsisiDm-*) were recorded but low grade silvering of yellow was often not noted at the time. One grandparent was certainly and the other probably *Sisi*. The gene *dm* was present in the ancestry but apparently produces no effect in *Cff* except in the combination *Cffsisidmdm*. Several other near-whites, all *eeCF-sisidmdm* segregated out in related matings, as well as silvered yellows *eeCffsisiDm-*.

Table 11 shows the frequency of self white (at birth) and near-whites with sepia or yellow on the head, according to probable *c* genotype, among the progenies in which all self whites could be attributed to *sisidmdm*. It appears that about 52 percent of *sisidmdm* with *C*, *c$^k$c$^k$*, or *c$^d$c$^d$* (in which yellow as well as sepia head spots should almost always be apparent at birth), showed such spots, leaving 48 percent self white. Among those of genotypes *c$^k$c$^a$*, *c$^d$c$^r$*, and *c$^d$c$^a$*, in which pale cream head spots are hardly distinguishable at birth, only about 21 percent were recorded as having head spots. This could mean that the frequency of potentially spotted areas is actually much reduced in the latter, but it is more

TABLE 11

*Self white (at birth), sepia spot and yellow spot among animals from matings of Type A. The* c *genotypes apportioned in segregating litters as described in the text*

| | *sisi dmdm* | | | | | |
|---|---|---|---|---|---|---|
| | Self white | Sepia spot | Yellow spot | Total | Percent with spot | |
| $C$ | 8 | 5 | 6 | 19 | 57.9 | |
| $c^kc^k$ | 1 | 0 | 1 | 2 | 50.0 | 52.2 |
| $c^dc^d$ | 13 | 1 | 11 | 25 | 48.0 | |
| $c^kc^a$ | 4 | 0 | 3 | 7 | 42.9 | |
| $c^dc^r$ | 12 | 1 | 3 | 16 | 25.0 | 21.3 |
| $c^dc^a$ | 43 | 6 | 3 | 52 | 17.3 | |
| $c^rc^r$ | 0 | 3 | 0 | 3 | 100.0 | |
| $c^rc^a$ | 3 | 5 | 0 | 8 | 62.5 | |
| Total | 84 | 21 | 27 | 132 | 36.4 | |

probable, in view of the considerable proportions of surviving pure whites that later were found to have very pale head spots, that the percentage with potential spots is independent of the *c* genotype and that the amount of pigment developed in those with lower *c* genotypes is usually almost or quite imperceptible.

### *Differential mortality*

The experiments with silver-whites were frustrated unduly often by early death. Table 12 shows the numbers born dead, that died before weaning, and that reached weaning among identifiable *sisidmdm*, unclassifiable self-whites and non-whites in all litters that contained the first. There is clearly heterogeneity with respect to mortality ($\chi^2 = 22.7$, n = 4).

Upon comparing merely the unclassifiable whites (largely albinos) and the non-whites, no significant difference is found ($\chi^2 = 4.4$, n = 2).

TABLE 12

*Number of identifiable silver-whites* (sisidmdm), *of pink-eyed white littermates (largely albinos* $c^ac^a$, *some* sisidmdm), *and of non silver-white littermates born dead, dying between birth and weaning, and raised to weaning*

| | Born dead | Died | Raised | Total |
|---|---|---|---|---|
| Silver-white | 43 | 40 | 69 | 152 |
| Pink-eyed white | 22 | 4 | 54 | 80 |
| Non silver-white | 51 | 28 | 125 | 204 |
| Total | 116 | 72 | 248 | 436 |

Upon comparing those born alive with those born dead, there is no significant difference whether the unclassifiable whites are combined with the non-whites ($\chi^2 = 0.5$, n = 1) or omitted ($\chi^2 = 0.3$, n = 1).

On considering only those born alive a highly significant difference is found between those that died before weaning and those that were raised. This holds if the unclassifiable whites are combined with the non-whites ($\chi^2$=17.9, n=1) or omitted ($\chi^2$=10.3, n=1), 85.5 percent of the non-whites were raised of those born alive but only 60.7 percent of those definitely *sisidmdm*. It is clear that there is no appreciable differential mortality up to birth but that the viability of *sisi dmdm* is definitely lower than normal thereafter.

### *Sterility of male silver-whites*

In the course of experiments intended to multiply or to test the silver-whites, it became apparent that no offspring were being obtained from males that were unequivocally of this type. In estimating the effective duration of matings, these were treated as starting with the month in which the male reached four months of age (average 3.5 months) or with the month following that in which the mating was made, if the male was already more than three months old at mating. The mating was treated as continuing through the month in which the female died or was disposed of. The first litter is usually produced so promptly (after a gestation period of about 68 days), that nonproductive matings of even three months duration by the above reckoning are uncommon among normal animals. The females referred to in this section were not silver-whites unless so stated (two cases).

The eighteen mated non silver-white brothers of silver-white males were all fertile and produced 190 litters in 281 months of mating on average of 3.84 per year (Table 13).

It became apparent that the testes of silver-white males remained abnormally small. These were dissected out and weighed in a number of cases as noted below. In three fertile non silver-white males, the combined weights of the testes including the epididymides were 5.3, 6.0, and 7.2 grams. The first was sepia 11-yellow 3, $c^dc^a$++*dmdm*, the second a very strong silver (grade 11), from RoXR, with a poor breeding record (three litters in 18 months), and the last a brown that produced only one litter near the beginning of nine months of mating. In three apparently unproductive males that were not silver-whites (duration of matings—3, 4, and 7 months) the testes plus epididymides weighed 5.0, 5.8, and 6.0 grams. The first was a silver, grade 5 ($c^dc^d$ *sisi* ++), the second a black ($C$ + *si* + *dm*) and the third a black with silvering of grade 7 ($C$ *sisi* ++). The average for all six not *sisidmdm* is 5.9 grams. In all of these, the epididymides were white and distended with sperm.

Three black-eyed white males produced no young in matings of 5, 6 and 6 months duration. In one (♂ 4781, discussed later) the combined weight of the testes was 1.95 grams. The epididymides were glassy and empty. In two unmated black-eyed whites (♂ 6756 and ♂ 6798, discussed later) single testes weighed only 0.40 and 0.25 grams. Three black-eyed near-white males produced no litters in 8, 8 and 14 months. In one, the combined weight of testes and epididymides was 1.0 grams. The latter were again glassy and empty. Seven pink-eyed near-whites produced no litters in 5, 6, 7, 7, 13, 21 and 21 months. The testes were dis-

TABLE 13

*Productivity of silver-white males and of their brothers (all fertile) with non silver-white females, and of silver-white females and of their sisters (all fertile) with non silver-white males. Thirteen of the males (first row) were definitely* sisidmdm *by parentage and color, four (second row) were pink-eyed whites that could be* sisidmdm *or merely* $c^ac^a$ *by color and parentage but are believed to be* sisidmdm *by their sterility and (in two cases) diminutive testes. The silver-white females were all definitely* sisidmdm. *They are divided according to apparent sterility or fertility. BA=born alive, RBA=raised of these born alive and R=raised*

| Test parent Sex | Genotype | Duration No. | Mo. | Mo./ind. | Litters (L) No. | L/year | Offspring (O) No. | O/L | O/year | Fate %BA | %RBA | %R | R/yr. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ♂ | *sisidmdm* | 13 | 127 | 9.8 | 0 | 0 | 0 | ... | 0 | ... | ... | ... | 0 |
| ♂ | *sisidmdm* ($c^ac^a$ possible) | 4 | 27 | 6.7 | 0 | 0 | 0 | ... | 0 | ... | ... | ... | 0 |
| ♂ | *sisidmdm* total | 17 | 154 | 9.1 | 0 | 0 | 0 | ... | 0 | ... | ... | ... | 0 |
| ♂ | not *sisidmdm* (brothers of tested ♂♂) | 18 | 281 | 15.6 | 190 | 3.84 | 345 | 3.83 | 14.7 | 70.7 | 79.9 | 56.5 | 8.33 |
| ♀ | *sisidmdm* (sterile) | 10 | 69 | 6.9 | 0 | 0 | 0 | ... | 0 | ... | ... | ... | 0 |
| ♀ | *sisidmdm* (fertile) | 11 | 91 | 8.3 | 28 | 3.69 | 79 | 2.82 | 10.4 | 63.0 | 87.0 | 54.8 | 5.52 |
| ♀ | *sisidmdm* total | 21 | 160 | 7.6 | 28 | 2.10 | 79 | 2.82 | 5.9 | 63.0 | 87.0 | 54.8 | 3.08 |
| ♀ | not *sisidmdm* (sisters of tested ♀♀) | 26 | 261 | 10.0 | 84 | 3.86 | 287 | 3.42 | 13.2 | 74.4 | 84.0 | 62.5 | 8.25 |

sected out in two. The combined weight with epididymides were 1.3 and 1.6 grams. In these also, the latter were glassy and empty. Five pink-eyed self whites from matings that produced silver-whites, produced no young in 3, 4, 6, 7 and 10 months. None of these was necessarily *sisidmdm* since two were certainly and three possibly albinos, $c^a c^a$. The mating of only three months duration was with a *sisidmdm* female and may have been unproductive for this reason. In two of the others, the testes and epididymides were weighed, the totals being 1.2 and 1.7 grams and the condition of the epididymides as before.

The average weight for the six unproductive whites or near-whites is 1.3 grams (and single testes of two other silver-whites averaged only 0.3 grams) in contrast with 5.9 grams for both testes plus epididymides of six not *sisidmdm*.

There was a considerable number of pink-eyed self whites from matings that produced undoubted *sisidmdm* that were fertile but these could all have been merely albinos. One pink-eyed self white from such a mating produced no litters in three months with a female known to be fertile, but is believed to have been merely an inadequately tested ordinary albino since the weight of the testes and epididymides was 5.0 grams and the latter were white and distended as in the control males. A white male with sepia-cream head spot produced no young in five months but had normal testes and white, distended epididymides, total weight 5.0 grams. The absence of young in this case was undoubtedly due to the female. She was a sister, undoubtedly $c^d c^a$*sisidmdm* (white with a small sepia head spot of grade 5), ovaries cystic, uterus grossly enlarged (2 cm in diameter, horns each 1.2 cm in diameter, lumen very fine). The head spots in the male in contrast were no lighter than in other low grade dilutes produced by this and other matings (sepia 12-cream 2). It was moreover evident from other offspring from the mating (L348) that high grade white spotting was segregating. It seems reasonably certain that this was merely an example of high grade spotting, $c^d c^a$ *ssSisidmdm*. The only other tested male (four litters in 18 months) which had been thought to be possibly a silver-white was a white with large sepia (grade 10) spots on both sides of the head. His parents (brother-sister) were siblings of two undoubted silver-whites. None of the seven other offspring were silver-whites but one showed moderately high grade spotting. Again the most probable interpretation is that this male was a high grade spotted segregant of the extreme dilute type, $c^d c^a$*ssSisidmdm*. Fortunately high grade spotting segregated out in only a few of the matings, and there were very few whites or near-whites about which there was any serious doubt on this account.

Histologic and experimental studies of probable *sisidmdm* males were begun by PROFESSOR C. R. MOORE but were interrupted by his fatal illness. Following are the notes which he made on the three cases which he completed.

"♂ *4181 black-eyed white from mating W12, born April 5, 1952*

Jan. 16, 1953. Autopsy. Body weight 885 g.
Testis not palpable in scrotum. No evident pathology of viscera. Testis small, epididymis glassy. No spermatozoa present. Seminal vesicles well distended with secretion (indicates plenty of male hormone secretion).

Vas deferens on right entered middle of seminal vesicle instead of common ejaculatory duct (atypical).

Weight

| | |
|---|---|
| 2 testes (without epididymis) | 1.95 g. |
| 2 seminal vesicles | 3.15 g. |
| 2 adrenals | .76 g. |
| 2 thyroids | .20 g. |
| pituitary | .020 g. |

Testis slide failed to show any spermatogenetic activity. Single row of basal cells in seminiferous tubules. Interstitial tissue prominent and in general excessive. Thyroid and adrenal essentially normal histologically. Pituitary slides suggest some abnormality but not yet completely convinced that it is atypical histologically.

*♂ 6756 black-eyed white from L348, born Dec. 14, 1952*

a) May 4, 1953. Right testis removed; weight 0.40 gms. (slide 284A) given one guinea pig pituitary implant per day for 15 days.

b) No external signs of testis descent. Abdomen opened, small testicular biopsy taken through puncture through tunica albuginea (slide 284B). Then gave 14 daily subcutaneous injections of gonadal stimulating hormone (gonadogen-equine gonadotrophin, 10 rat units or 200 international units).

c) Autopsy June 1 (5½ months old)

Organs in general normal; adhesions had developed after operation between rough surface of testis and inside lining of body wall; testis could not have descended.

At autopsy

| | |
|---|---|
| body weight | 460 gm. |
| left testis (earlier biopsy) | 0.72 g. (slide 284C) |
| 2 seminal vesicles | 2.7 g. plenty of secretion |

Testis slide 284A (before treatment) no spermatogenesis; single basal row of cells in seminiferous tubules.

Testis slide 284B (biopsy after 15 pituitary implants) no appreciable change from untreated.

Testis slide 284C (15 pituitary implants then 14 daily gonadotrophin injections). Testis larger than original one (heavier) but no improvement in spermatogenesis; interstitial tissue greatly stimulated.

*♂ 6798 black-eyed white from L350, born Dec. 18, 1952*

May 4, 1953. Testes not palpable. Body weight 315 gm. Age 4½ months. Right testes removed: weight 0.25 gm. Slide 285A. Injected testosterone propionate, 0.5 mgs daily, 14 days. No external signs of change. Then testosterone propionate increased to 2.5 mgm daily for 14 days. (Slide 285B)

Autopsy June 1. Body weight 340 gm.

All organs normal in appearance except testis. Testis high in abdomen (could have descended). Left testis weight 0.17 gm., 2 seminal vesicles 1.17 gm. distended with secretion. Slide 285B (left testis following 14 days injection of testosterone propionate 0.5 mgm daily, then 14 days, 2.5 mgm daily) does

not show appreciable change from right testis removed before treatment. These dosages of male hormone did not stimulate spermatogenesis."

From these cases it appears that there is a primary failure of spermatogenesis, due presumably to absence of spermatogonia. There is no evidence of deficiency of interstitial cells in the testis or of male hormone, nothing abnormal in the histology of the adrenals or thyroid, nothing clearly atypical in the pituitary and no indication of spermatogenesis by pituitary implants, equine gonadotrophin or testosterone propionate.

Table 13 gives a summary of the breeding records of the 13 males believed to be *sisidmdm* by color and ancestry, the four pink-eyed whites, probably *sisidmdm* by color, ancestry and record, and the 18 brothers definitely not *sisidmdm*, all mated with normal females.

### *Low productivity of female silver-whites*

One case of abnormality in the reproductive tract of an *sisidmdm* female has been referred to. No gross abnormalities were found in a number of other cases, however, and many were fertile—yet productivity was clearly subnormal. The most important results are summarized in Table 13 in which the records of the sisters of the tested *sisidmdm* females are used as controls. Duration is calculated as described for the tests of males. The shortest durations entered were of three months for each of two *sisidmdm* females one with one litter, one with none, and of two months for each of two controls, each with one litter. In the cases of two silver-whites, litters of three each were removed before birth, but close to term, and a litter of two, near term, from a control female, at the time of disposal of the guinea pig colony. As these could not be included in the categories born alive (BA), raised of those born alive (RBA) or raised (R), duration is shortened by two months in each case before calculating the number of young raised per year. Only matings with normal males are considered.

Among the 21 silver-white females, ten produced no litters although mated on the average 6.9 months each. All of the 26 normal sisters were fertile. It should be said that there was one sister, not included, that produced no litter in ten months but in this case the male produced no litter in 10 months with another normal female and was thus probably responsible for the failure. This difference in fertility between *sisidmdm* and their sisters is highly significant ($\chi^2 = 12.7$, one degree of freedom).

Considering only the fertile matings, there is little difference in the number of litters per year, 3.69 for the silver whites *vs.* 3.86 for their normal sisters. There is a greater difference in mean size of litter, 2.82 for *sisidmdm vs.* 3.42 for their sisters but the significance is doubtful (standard deviation 1.42 for silver-whites, 1.49 for the controls, $t = 1.90$). A considerably smaller percentage of the offspring of silver-whites were born alive (63.0 percent *vs.* 74.4 percent) but a slightly larger percentage of those born alive were raised (87.0 percent *vs.* 84.0 percent). The percentage raised of all young (product of preceding percentages) was less for silver-whites (54.8 percent *vs.* 62.5 percent), but not significantly. The number of offspring raised per year, the over-all estimate of reproductive

efficiency, is much smaller for the silver-whites (5.52 *vs.* 8.25) but the standard deviations are such (4.20 for silver white, 4.49 for the controls) that the difference is only 1.77 times its standard error.

If, however, records of all mated silver-white females are compared with those of their normal sisters, the discrepancy becomes much greater because of the large proportion of the former that were apparently sterile. The average number of litters per year becomes 2.10 for the silver-whites as opposed to 3.86 for their sisters, the offspring per year becomes 5.93 *vs.* 13.20 and the number raised per year 3.08 *vs.* 8.25.

It may be seen that the records of the normal brothers of the tested silver-white males and those of the normal sisters of tested silver-white females were rather similar (except in average duration).

### *Anemia of silver-whites*

I am indebted to Dr. W. K. Silvers for determinations of hemoglobin in a number of silver-whites and of non silver-whites from the same or closely related matings. Two determinations were made for each guinea pig (average difference 26.2 on the scale used). The average for seven control guinea pigs was 606.4, for 10 silver-whites 456.1, giving a difference of 150.3 with a standard error of 16.2. The difference is thus highly significant (9.3 SE).

Three of the controls were males (average 631.3) and four females (average 595.2). Two of the silver-whites were males (average 445.5) and eight females (average 458.7). Thus there is no consistent sex difference with the numbers tested.

Paired comparisons could be made on the basis of six matings represented by both silver-whites and controls. Two silver-whites from one mating and two controls from another are averaged in each case. Three of the silver-whites had no controls from the same mating and are omitted. The difference, 146.2 is 6.7 times its standard error which is significant at the .001 level. All of the matings were so closely related, however, that the unpaired comparison may be taken as valid.

Thus silver-whites have only about 75 percent of the hemoglobin concentration of their normal relatives. This may contribute to the low viability and low productivity of females of genotype *sisidmdm*. It can hardly have any direct relation to the absence of spermatogenesis in the males.

### *Possible earlier references to* dm

The guinea pig colony in which the present data were obtained traces largely to a colony of the Bureau of Animal Industry, U.S. Department of Agriculture (which contained the color factors $e^p$, *e; A, a; B, C,* $c^k$, $c^a$; *P, F, f; S, s; Si, si* and *Dm;* and a few animals kindly presented by Dr. W. E. Castle in 1916 which introduced *E, b,* $c^d$, $c^r$, *p* and almost certainly *dm*. The initial intensity of $c^d$ genotypes, probably associated with *dmdm*, was increased by matings with albinos of the U.S.D.A. stock though not to such a degree that there was overlap of $c^d$ sepias with $c^k$ sepias. An association of $c^d$ with dilution modifiers persisted for a long

time in certain largely isolated strains (cf. WRIGHT 1949, p. 254). It now appears that this was due to the gene here designated *dm*.

CASTLE (1912) described an experiment in which he selected for extreme dilution in a strain of light sepias, creams and albinos by mating the lightest sepias and lightest creams. One of the matings produced an animal with a white coat except for a spot on the right side of the head and a small one on the body of a very pale color, described as like that of a pink-eyed (*pp*) mouse. Gene *p*, it may be noted, was not introduced into the colony until a year later. The eyes were described as pink but with a faintly pigmented streak in the iris.

This is like a silver-white of genotype $Eec^dc^asisidmdm$, except that the eyes of the latter, while lighter (dark red) than the black eyes of sepia or cream litter mates would not be described as pink. Silvering was present in other strains in DR. CASTLE's laboratory. If *si* and *dm* were present at all in the foundation stock of the section experiment, individuals of genotype (*Ee* or *ee*)$c^dc^aSisidmdm$ would tend to be selected for mating because of their extreme dilution and should have produced some silver-whites. Other factors for dilution assembled by the selection, but not present in my colony, might have been responsible for the greater dilution of eye color than that which I have observed in silver-whites.

In a later paper (1916) CASTLE described crosses between the Peruvian cavy (*Cavia cutleri*) believed to be the wild ancestor of the guinea pig, and animals of two guinea pig strains. *Cavia cutleri* is paler than a cream agouti guinea pig. "The fur is agouti-ticked and the belly light, but the yellow of the ticking and belly is so pale as to resemble a dirty white or very pale cream shade." On crossing with albinos from an otherwise very intense black strain (race B: *E aa BB* $c^ac^a$ *PP FF*) the offspring were intense agoutis (with red ticking and belly) as intense as those produced from crosses with blacks of the same strain. It was concluded that *cutleri* transmitted gene *C* and must owe its dilution to factors independent of the *c* series, which had just been described (WRIGHT 1915). As none of the 58 $F_2$ animals was as light as the *cutleri* grandparent, it was also concluded that "the darker coloration introduced by the cross, apparently through the albino parent, does not behave as a simple Mendelian character, either dominant or recessive."

The other strain with which the wild cavies were crossed was one (race C) in which the animals were either very pale brown-eyed creams (*ee aa bb* $c^dc^a$ *PP FF*) or the corresponding albinos ($c^ac^a$). The hybrids from either the creams or the albinos, while more intense than either the wild cavies or the creams, were "much lighter than the hybrids produced in the cross with race B albinos." Among 81 $F_2$ young from $F_1$ pairs that produced albinos ($Cc^a \times Cc^a$), 11 were very pale. It appears that race C has one or more dilution factors in common with *cutleri*, absent in race B, as well as intensity factors, probably in common with race B, but replaced by dilution factors in the wild species. As race C seems to have been the source of *dm* of our colony the question is raised whether *dm* may not be present in the wild species and thus the type allele at its locus. As *dm* has no appreciable effect in our colony in the presence of *C* (except in association with *sisi*) it must be supposed that the wild species differs from race C at some other locus or loci responsible jointly with *dm* for its dilution in the presence of gene *C*. This can hardly be *si* in view of the self white or near-white character of *sisi*

*dmdm*, as well as the sterility of males of this genotype. If we let *dc* represent such a gene in *Cavia cutleri*, the hypothesis in its simplest form is as follows:

| | | |
|---|---|---|
| *Cavia cutleri* | *CC dmdm dcdc* | very dilute |
| Race C, cream | $c^d c^a$ *dmdm DcDc* | very dilute |
| $F_1$ (*cutleri* × race C albino) | $Cc^a$ *dmdm Dcdc* | moderately dilute |
| Like race C except C for $c^d$ | *CC dmdm DcDc* | intense |
| $F_1$ (*cutleri* × race B albino) | $Cc^a$ *Dmdm Dcdc* | intense |
| Race B black | $Cc^a$ *DmDm DcDc* | very intense |

The failure to recover the dilution of *cutleri* in 58 $F_2$'s from the cross with race B albinos, indicates that it must differ in one or more additional factors. The segregation of only 11 pale-colored in 81 $F_2$ hybrids with race C albinos, probably indicates at least one additional factor of type *dc* in *cutleri*.

IBSEN (1932) listed the variations from the typical colors associated with combinations of major color factors that were present in his colony and assigned genic symbols in each case. He implied, however, that adequate data had not been obtained in all cases. No data were given and it is not clear which ones were considered to be demonstrated. He described extreme dilution of dilute yellow (*ee* $c^d c^a$) as due to a recessive gene *li*. This might have been merely an effect of *si* (almost certainly the same as his Ro, studied by IBSEN and GOERTZEN (1951) on a black background, and thus present in the colony). If not, it is possible that our *dm* is the same as his *li*. There is the apparent difference that *li* is stated to be recessive while *Dmdm* is as intermediate as *Sisi*. There is also the apparent difference that no effect on sepia (or brown) is ascribed to *li*, while such an effect (in tortoise-shells for example) is obvious in the case of *dm*. It is not stated, however, that effects were looked for on any other than a yellow background. It is also quite possible that there is both a specific modifier of light yellow (*li*) that was present in his stock but not mine and a general modifier of pigmentation (*dm*) with a peculiar interaction effect with silver that was present in my colony but not his.

In a brief reference in a previous paper (1956), the symbol *di* was used for the gene that is here called *dm*, overlooking the fact that IBSEN had assigned the symbol *di* to a wholly different variation, dilution at the base of black hair, in his 1932 paper.

## SUMMARY

The first order variations in intensity of dark eyed sepias in the guinea pig colony under consideration were due to the *c* series of alleles (*C*, $c^k$, $c^d$, $c^r$, $c^a$). Those in yellow are due to the *c* series and *F*, *f*. Most of the residual variability in sepia and yellow must have been due to common factors in view of the strong correlation between the intensities of sepia and yellow segregants, or within tortoise-shells, of the same *c* genotype (and *FF*). Most of the residual variability of pink-eyed pale sepias (*pp*) was independent.

Most of the residual variability in intensity of dark eyed sepia and yellow depended on two genes silvering (*si*) and diminished (*dm*). The former tends to produce silvering (sprinkling with white), blotching (irregularity in degree of

silvering even in symmetrically placed areas) and dilution. The silvering and blotching are most conspicuous with higher *c* genotypes, (especially with *C* in blacks, reds and pink-eyed pale sepias), the dilution in lower ones (especially $c^d c^a$ in which dilution, uniform except for light belly, largely replaces silvering in both sepias and yellows). The gene *dm* has somewhat similar effects with lower *c* genotypes, but no silvering effect and no effect with *C* except in *sisi dmdm*.

The dilution effects of the loci act cumulatively to produce extreme dilution in *Sisidmdm* and *sisiDmdm*.

In the combination *sisidmdm* the coat is usually wholly white except (in about 50 percent) for one or two small spots of pale color on the cheeks. With *C*, these spots are strongly silvered. With $c^d c^a$ they are so pale as to be easily overlooked at birth. Eye color (if *P* is present) is slightly reduced.

Silver-whites (*sisidmdm*) have a higher mortality rate after birth than their normal litter mates. The males are sterile as far as known. Those examined have testes of about 25 percent normal weight.

Spermatogenesis was found to be wholly absent in those examined and could not be induced by testosterone propionate, pituitary implants, or gonadotrophin. Interstitial tissue and amount of male hormone seemed normal. (C. R. Moore).

About 50 percent of the silver-white females have been sterile. The others are probably somewhat low in productivity.

Silver-whites have about 75 percent of the hemoglobin concentration of normals of the same strain. (W. K. Silvers).

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# A CHROMATOGRAPHIC ANALYSIS OF THE FREE AMINO ACIDS IN THE BLOOD PLASMA OF THREE INBRED STRAINS OF THE HOUSE MOUSE, MUS MUSCULUS[1]

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PHYSIOLOGICAL differences between animals may be correlated with variations in the free amino acid level of tissues and fluids. This is illustrated by the results of research by DENT (1947) on the amino-aciduria associated with the Fanconi syndrome. An increased level of some amino acids was found chromatographically in the urine of persons affected by this disorder. WILLIAMS *et al.* (1951) searched for urinary amino acid metabolic patterns associated with human disease. They developed methods of paper chromatography and applied them in studies of urinary amino acids in rats, in unrelated normal human beings, and in unrelated human beings suffering from schizophrenia, mental deficiency, and overweight and underweight. Differences in the free amino acid content of the blood of seven species of insects have been reported by PRATT (1950). Similar studies using paper chromatography have shown biochemical differences between normal and tumorous tissues in mice (ROBERTS and FRANKEL 1949; MICKELSON and BARVICK 1956) and between growing and regressing tumors (ROBERTS and BORGES 1955). Thus chromatographic techniques have been useful in discovering differences between species of insects, between unrelated human beings with respect to metabolic and mental disorders, and between normal and abnormal tissues within an inbred strain of mice.

BERRY (1953) reported a genetic interpretation of the variations he observed in urinary excretion patterns found in a human population in Texas. A chromatographic pattern difference between male and female Drosophila which was evident under ultraviolet light was reported by BUZZATI-TRAVERSO (1953a). Genetic control of conditions such as cystinuria (DENT and HARRIS 1951), and of variations in the quantity of particular substances in the tissues and fluids (CRUMPLER *et al.* 1951) have been found by similar chromatographic methods. SUTTON and CLARK (1955) found a genetically influenced lysine variation when comparing chromatographed urine of Chinese and Caucasoids. Differences in the excretion level of taurine, lysine, and methionine sulfoxide were found between inbred strains of rats (REED 1951). BERRY *et al.* (1955), in chromatographic studies on dizygotic and monozygotic twins, compared their intrapair variation in excretion

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rates of various amino acids, and associated these with genetic control. Specific genetic control has been shown for the excretion of beta-aminoisobutyric acid in human beings (HARRIS 1953). HARRIS suggests that a high excretion of this acid results from the presence of a homozygous recessive genotype. BUZZATI-TRAVERSO (1953b) was able to identify Drosophila heterozygotes carrying the recessive conditions bent, eyeless, shortvein, spineless-aristapedia, and vestigial, by their amino acid patterns. He obtained consistent patterns "with a high degree of independence of feeding and other environmental conditions."

Because of results such as those described above, this study was undertaken to search for consistent differences between the amino acid contents of the blood plasmas of highly inbred strains of mice. Differences between strains as determined by the statistical analyses which follow, may be attributed to differences in the genetic background of the strains. Differences within a strain may be attributed to the effects of segregating genes or to sexual differences. Three inbred strains suitable for this study were present in the stock maintained by the Genetics Section of the Department of Zoology and Entomology of the Ohio State University. They were SEC/2 Gn-*d*, CBA/Ca-*se*, and Furless. The genes for dense/dilute coat color in the first strain, the genes for short ear/normal ear in the second strain, and the genes for furless/normal coat in the third strain are segregating. Since these genes are associated with physiological processes involved in the synthesis of highly proteinaceous structures, detectable amino acid differences might be expected to occur in the blood. By utilizing chromatographic methods to determine amino acid levels, some insight was gained into the action that the particular gene segregating in each strain has upon various physiological processes. Statistically significant differences in amino acid content were shown: (1) between the genotypes within two of the three strains of mice tested, (2) between the sexes within each of the three strains tested, and (3) between the three strains tested.

## MATERIALS AND METHODS

Mice of three different inbred strains were used: The SEC/2 Gn-*d* strain with the genes for dense/dilute (*D*/*d*) coat color segregating; CBA/Ca-*se* strain with the genes for normal ear/short ear (*Se*/*se*) segregating; and the Furless strain with the genes for normal/furless (*Fs*/*fs*) coat segregating. Equal numbers of mice were used of (a) each genotype of the gene segregating in that strain, (b) of each sex, and (c) of each strain. In this way differences in concentration of each amino acid were compared between strains, between sexes within strains, and between genotypes within strains.

All the mice of a particular strain that were used in the tests were the descendants of one pair of mice of that strain. Two sublines were set up in each strain from the second generation offspring of this initial pair. One subline was composed of mice of the genotype *AA* mated to *AA* mice. (*A* and *a* are used here as general symbols for the three segregating loci.) The other subline was composed of mice of the genotype *Aa* mated to *aa* mice. The genotype of each *AA* or *Aa* mouse used in matings was determined by progeny testing. After three or four

generations of brother-sister mating of progeny-tested mice, there were enough mated mice of the same generation of each subline to produce the number of mice necessary for the testing procedure.

The mice of the Furless strain which provided blood samples were in the 15th generation of brother-sister mating, those of the CBA/Ca-*se* strain in the 24th generation, and those of the SEC/2 Gn-*d* strain in the 45th generation. Thus the percentage of homozygous loci not linked to the gene segregating in each particular strain should exceed 97 percent in the Furless strain, and approach 100 percent in the SEC/2 Gn-*d* strain and the CBA/Ca-*se* strain (WRIGHT 1921).

The strain, sex, and genotype of each mouse was recorded so that differences between strains, differences between sexes, and differences between genotypes within a strain could be tested. The term "strain," as used here, is defined as an equal number of male and female mice of each of the three possible genotypes of the segregating locus.

It was necessary to foster all Furless and CBA/Ca-*se* litters on SEC/2 Gn-*d* mothers to get the proper mice at the proper time since certain mothers were neglecting their litters. All pregnant females were checked daily, and each new born litter was fostered onto an SEC/2 mother which had borne a litter within the previous few days. By this means, the necessary mice were obtained at the proper time. All mice, fostered and nonfostered, were subsequently treated in the same manner.

The young mice were weaned at four weeks of age and the males and females put into separate cages. When the mice were six weeks of age, they were deprived of food for 12 hours and bled of 500 microliters of blood by heart puncture. The free amino acids present in the blood plasma were separated in alcohol, concentrated in a vacuum oven, and spotted on a sheet of filter paper.

Two dimensional paper chromatograms were run on each of four replicates of every combination of genotype, sex, and strain. The chromatographic method was based on that reported by THOMPSON and STEWARD (1951). Water saturated phenol was used as solvent in the first dimension, and a 1:3 mixture of 2,4,6-trimethylpyridine (collidine) and 2,4-lutidine was saturated with water and used as the solvent in the second dimension. The developing reagent was a 0.2 percent solution of ninhydrin in 95 percent ethanol containing 0.4 percent of the 1:3 collidine-lutidine reagent mentioned above.

The amino acids present on the test chromatograms were tentatively identified by their position and color, using information given by DENT (1948). The spot was considered to be identified when the addition of a quantity of known amino acid to the test sample to be chromatographed intensified the spot in question (reinforcement). After all the spots had been identified a master chromatogram was drawn and all spots labeled. The spots on the experimental chromatograms were identified by relative position and color with reference to the master chromatogram. The spots were eluted from the developed chromatograms and were analyzed spectrophotometrically. The resulting measurements of optical density were converted to proportions of total optical density.

The data for each amino acid obtained by this procedure were analyzed for differences between genotypes and between sexes within each strain by a two way analysis of variance, and for differences between the three strains and between the sexes by another two way analysis of variance.

## RESULTS AND INTERPRETATIONS

### *General considerations*

The developed chromatograms revealed 16 spots, each of which was found to be caused by one or more of the following substances; glutathione, aspartic acid, homocysteine, cystine, taurine, glutamic acid, serine, glycine, lysine, arginine, glutamine, alpha alanine, beta alanine, valine, norvaline, isoleucine, and leucine (Figure 1). Due to the overlapping of positions taken by valine and norvaline and by isoleucine and leucine, they were each measured as one spot. The spots resulting from cystine and from taurine were always relatively large and diffuse. It was often difficult to determine the exact extent of either of these two spots. The serine and alpha alanine spots often ran together, as did those for glycine and glutamine. The glutamine and beta alanine spots invariably overlapped at their edges. Whenever a reserve chromatogram of a mouse of the same strain, sex, and genotype was available, it was processed in place of one in which the spots had run together. Spots of glutathione, aspartic acid, homocysteine, glutamic acid, lysine, arginine, valine-norvaline, and isoleucine-leucine were usually either dense and well defined, or if weak were separated from the others well enough to be readily determined. Aspartic acid split into two well defined spots separated by the homocysteine spot. The two aspartic acid spots were eluted together and measured spectrophotometrically as one.

### *Statistical analysis*

Variations in the total optical density were observed between replicates of the same group. When all four replicates of a group were ranked 1, 2, 3, 4, for each amino acid, according to the magnitude of the optical density measurement, it was noted that the replicate with the highest total optical density was usually ranked first for each individual amino acid. The replicate with the lowest total optical density was usually ranked fourth. This means that either the free amino acid base level varies between individuals, or the starvation period was not long enough to bring the blood amino acids to the base level, or the chemical processing method was not standardized to the desired extent. If the optical density data were analyzed directly, the variation between replicates would appear in the source of variation labeled as the "error mean square." This would effectively prevent many, if not all, actual differences from being discovered. In other words the power of the tests would be very low. Therefore, the optical density readings were converted to proportions of the total optical density for that individual replicate. In this way all the replicates were put on the same base total, namely 1.0, and each individual reading was a portion of this such as .082, or .136. This

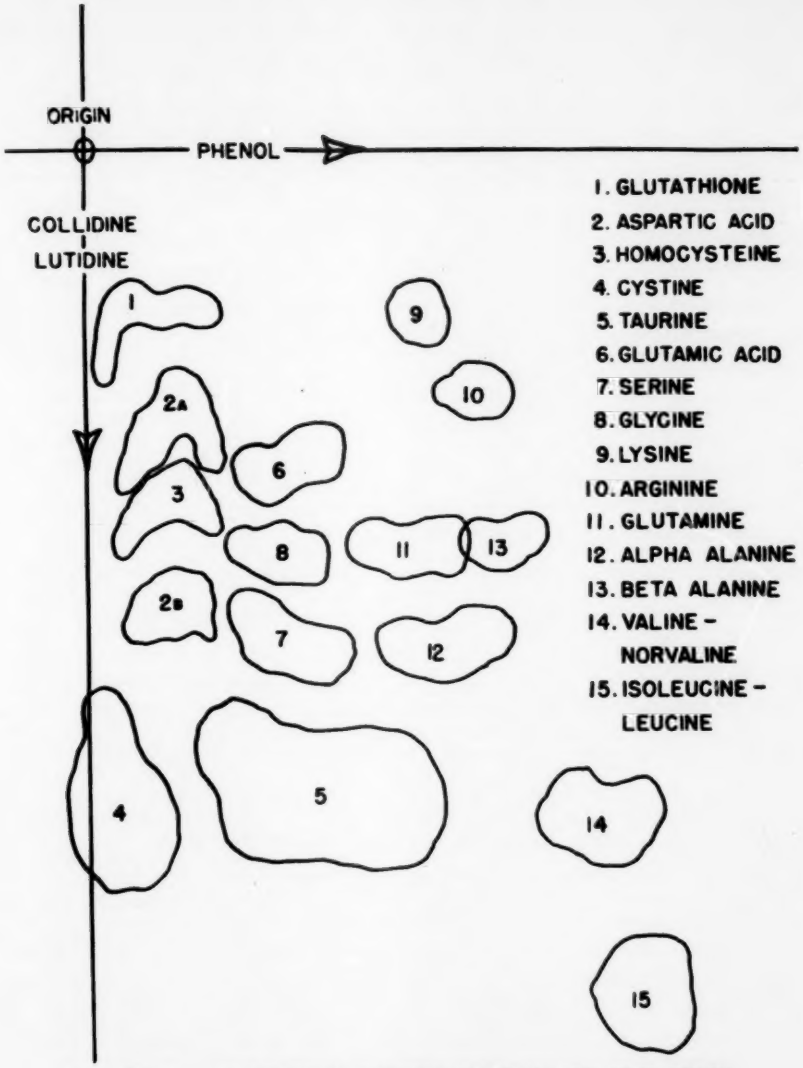



FIGURE 1. A TYPICAL CHROMATOGRAM OF THE FREE AMINO ACIDS IN MOUSE BLOOD

is the way the data have been reported in Tables 1 through 3. For convenience in calculating, however, all readings were multiplied by 1000, thus making each a whole number.

TABLE 1

*Mean proportion of individual amino acids, as converted from optical density measurements on chromatograms of the blood of the four replicate male and female mice of the* DD, Dd, *and* dd *genotypes in the SEC/2 Gn-d strain*

| Genotype | *DD* | | *Dd* | | *dd* | |
|---|---|---|---|---|---|---|
| Sex | M | F | M | F | M | F |
| 1. Glutathione | .091 | .118 | .084 | .085 | .095 | .081 |
| 2. Aspartic acid | .138 | .193 | .152 | .162 | .152 | .147 |
| 3. Homocysteine | .052 | .056 | .055 | .055 | .061 | .058 |
| 4. Cystine | .061 | .048 | .065 | .056 | .065 | .065 |
| 5. Taurine | .088 | .066 | .080 | .087 | .070 | .085 |
| 6. Glutamic acid | .062 | .052 | .053 | .053 | .048 | .046 |
| 7. Serine | .055 | .052 | .061 | .056 | .050 | .061 |
| 8. Glycine | .073 | .054 | .074 | .069 | .057 | .059 |
| 9. Lysine | .031 | .025 | .028 | .039 | .029 | .031 |
| 10. Arginine | .030 | .026 | .038 | .032 | .037 | .027 |
| 11. Glutamine | .054 | .048 | .056 | .057 | .057 | .047 |
| 12. Alpha alanine | .079 | .084 | .066 | .071 | .058 | .068 |
| 13. Beta alanine | .025 | .024 | .028 | .024 | .038 | .026 |
| 14. Valine-norvaline | .069 | .077 | .075 | .072 | .097 | .083 |
| 15. Isoleucine-leucine | .093 | .080 | .084 | .086 | .089 | .120 |
| Sum | 1.001 | 1.003 | .999 | 1.004 | 1.003 | 1.004 |

TABLE 2

*Mean proportion of individual amino acids, as converted from optical density measurements on chromatograms of the blood of the four replicate male and female mice of the* SeSe, Sese, *and* sese *genotypes in the CBA/Ca-se strain*

| Genotype | *SeSe* | | *Sese* | | *sese* | |
|---|---|---|---|---|---|---|
| Sex | M | F | M | F | M | F |
| 1. Glutathione | .079 | .085 | .094 | .064 | .087 | .066 |
| 2. Aspartic acid | .155 | .153 | .146 | .122 | .157 | .106 |
| 3. Homocysteine | .081 | .082 | .072 | .052 | .068 | .048 |
| 4. Cystine | .065 | .058 | .058 | .071 | .068 | .083 |
| 5. Taurine | .104 | .071 | .081 | .110 | .078 | .104 |
| 6. Glutamic acid | .047 | .055 | .047 | .060 | .053 | .054 |
| 7. Serine | .049 | .052 | .050 | .060 | .046 | .056 |
| 8. Glycine | .056 | .056 | .051 | .065 | .055 | .052 |
| 9. Lysine | .032 | .044 | .040 | .051 | .042 | .045 |
| 10. Arginine | .024 | .038 | .031 | .042 | .026 | .044 |
| 11. Glutamine | .055 | .051 | .047 | .063 | .049 | .056 |
| 12. Alpha alanine | .069 | .074 | .068 | .084 | .071 | .071 |
| 13. Beta alanine | .023 | .031 | .035 | .030 | .026 | .036 |
| 14. Valine-norvaline | .079 | .073 | .094 | .067 | .084 | .087 |
| 15. Isoleucine-leucine | .085 | .079 | .089 | .063 | .093 | .093 |
| Sum | 1.003 | 1.002 | 1.003 | 1.004 | 1.003 | 1.002 |

TABLE 3

*Mean proportion of individual amino acids, as converted from optical density measurements on chromatograms of the blood of the four replicate male and female mice of the* FsFs, Fsfs, *and* fsfs *genotypes in the Furless strain*

| Genotype / Sex | *FsFs* M | *FsFs* F | *Fsfs* M | *Fsfs* F | *fsfs* M | *fsfs* F |
|---|---|---|---|---|---|---|
| 1. Glutathione | .104 | .091 | .096 | .112 | .114 | .136 |
| 2. Aspartic acid | .121 | .135 | .103 | .126 | .195 | .178 |
| 3. Homocysteine | .059 | .069 | .061 | .051 | .047 | .045 |
| 4. Cystine | .081 | .075 | .082 | .068 | .066 | .080 |
| 5. Taurine | .088 | .086 | .077 | .087 | .090 | .073 |
| 6. Glutamic acid | .050 | .054 | .061 | .043 | .049 | .028 |
| 7. Serine | .051 | .049 | .060 | .055 | .048 | .062 |
| 8. Glycine | .065 | .049 | .053 | .060 | .048 | .050 |
| 9. Lysine | .038 | .041 | .052 | .039 | .013 | .024 |
| 10. Arginine | .041 | .043 | .054 | .046 | .041 | .049 |
| 11. Glutamine | .065 | .055 | .047 | .056 | .051 | .053 |
| 12. Alpha alanine | .061 | .067 | .061 | .057 | .056 | .052 |
| 13. Beta alanine | .033 | .036 | .046 | .037 | .046 | .037 |
| 14. Valine-norvaline | .060 | .067 | .067 | .088 | .076 | .067 |
| 15. Isoleucine-leucine | .084 | .086 | .083 | .076 | .061 | .068 |
| Sum | 1.001 | 1.003 | 1.003 | 1.001 | 1.001 | 1.002 |

Readings of optical densities may be used for computations since THOMPSON and STEWARD (1951), by determining the optical density of spots which resulted from known concentrations of amino acids, have shown that a linear relationship exists between optical density and concentration. I verified these results by preparing similar standards.

The statistical analyses consist of a separate two way analysis of variance (sex by genotype) for each of the 15 amino acids in each of the three strains. In this way existing genotypic differences within a strain could be detected without being confounded with the genotypes of the other two strains. After these analyses a separate two way analysis of variance (sex by strain) was run for each of the 15 amino acids measured. This was done to detect differences between the three strains. The F values calculated from the data are considered to be significant if they exceed the tabulated F value for the appropriate degrees of freedom at the five percent level of significance (as listed in SNEDECOR's *Statistical Methods*, 4th Edition 1950). Significant F values are indicated in the tables by one asterisk (*). If the F value calculated from the data exceeds the tabular F value at the one percent level, it is marked by two asterisks (**). Summaries of all analyses appear in Tables 4a and b and 5 immediately following.

### *Biological interpretation*

Patterns of the amino acids were drawn, Figures 2 through 19 in Plates I, II, and III, and should be consulted for a visual picture of the differences mentioned in the following discussions of each amino acid. The patterns were modeled after

TABLE 4a

*Difference in amino acid levels between genotypes determined from two way analyses of variances within strains*

| Within strain | SEC | | | F Value | CBA | | | F Value | Furl. | | | F Value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *DD* | *Dd* | *dd* | | *SeSe* | *Sese* | *sese* | | *FsFs* | *Fsfs* | *fsfs* | |
| 1. Glutathione | H | L | M | 4.70* | H | M | L | < 1 | L | M | H | 5.36* |
| 2. Aspartic acid | H | M | L | < 1 | H | M | L | < 1 | M | L | H | 18.23** |
| 3. Homocysteine | L | M | H | < 1 | H | M | L | 3.30 | H | M | L | 1.90 |
| 4. Cystine | L | M | H | 1.30 | L | M | H | 1.08 | H | M | L | < 1 |
| 5. Taurine | = | H | = | 1.10 | L | H | M | < 1 | H | M | L | < 1 |
| 6. Glutamic acid | H | M | L | 1.84 | L | = | = | < 1 | = | = | L | 2.67 |
| 7. Serine | L | H | M | < 1 | L | H | M | < 1 | L | H | M | 1.00 |
| 8. Glycine | M | H | L | 4.26* | M | H | L | < 1 | = | = | L | 1.52 |
| 9. Lysine | L | H | M | 1.20 | L | H | M | 2.27 | M | H | L | 9.23** |
| 10. Arginine | L | H | M | 2.66 | L | H | M | < 1 | L | H | M | < 1 |
| 11. Glutamine | L | H | M | < 1 | = | H | = | < 1 | H | = | = | 2.43 |
| 12. Alpha alanine | H | M | L | 3.93* | = | H | = | < 1 | H | M | L | 1.86 |
| 13. Beta alanine | L | M | H | 1.76 | L | H | M | < 1 | L | = | = | < 1 |
| 14. Valine-norvaline | = | = | H | 4.51* | L | M | H | 2.98 | L | H | M | 3.42 |
| 15. Isoleucine-leucine | M | L | H | 3.17 | M | L | H | 1.48 | H | M | L | 4.64* |
| Five percent level of significance | | | | 3.55 | | | | 3.55 | | | | 3.55 |
| One percent level of significance | | | | 6.01 | | | | 6.01 | | | | 6.01 |

* Indicates significant at the 5% level.
** Indicates significant at the 1% level.
H Indicates high; M indicates medium; L indicates low; = indicates equal.

TABLE 4b

*Difference in amino acid levels determined from two way analyses of variances within strains (F value shown)*

| Within strain | Between sexes | | | Interaction (geno.-sex) | | |
|---|---|---|---|---|---|---|
| | SEC | CBA | Furl. | SEC | CBA | Furl. |
| 1. Glutathione | < 1 | 2.94 | 1.34 | 4.26* | 1.41 | 2.33 |
| 2. Aspartic acid | 2.55 | 2.63 | < 1 | 1.75 | < 1 | 1.37 |
| 3. Homocysteine | < 1 | 2.70 | < 1 | < 1 | < 1 | < 1 |
| 4. Cystine | 2.21 | < 1 | < 1 | < 1 | < 1 | 1.39 |
| 5. Taurine | < 1 | 2.15 | < 1 | 8.45** | 16.0** | 1.41 |
| 6. Glutamic acid | < 1 | 3.53 | 4.49* | < 1 | < 1 | 2.14 |
| 7. Serine | < 1 | 1.39 | < 1 | 1.61 | < 1 | 1.79 |
| 8. Glycine | 4.03 | < 1 | < 1 | 2.97 | < 1 | 2.52 |
| 9. Lysine | < 1 | 8.42** | < 1 | 2.49 | < 1 | 1.85 |
| 10. Arginine | 8.73** | 4.29 | < 1 | < 1 | < 1 | < 1 |
| 11. Glutamine | 1.34 | 1.83 | < 1 | < 1 | 1.74 | 2.71 |
| 12. Alpha alanine | 1.40 | 1.10 | < 1 | < 1 | < 1 | < 1 |
| 13. Beta alanine | 3.39 | < 1 | < 1 | 1.06 | < 1 | < 1 |
| 14. Valine-norvaline | < 1 | 8.72** | 2.14 | 1.39 | 7.09** | 4.26* |
| 15. Isoleucine-leucine | < 1 | 1.54 | < 1 | 3.33 | < 1 | < 1 |
| Five percent level of significance | | 4.41 | | | 3.55 | |
| One percent level of significance | | 8.28 | | | 6.01 | |

* Indicates significant at the 5% level.
** Indicates significant at the 1% level.

TABLE 5

*Difference in amino acid levels determined from two way analyses of variances between strains (F value shown)*

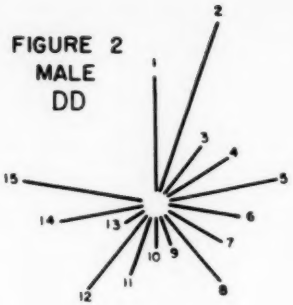
| | Between sexes | Between strains | Interaction (strain-sex) |
|---|---|---|---|
| 1. Glutathione | < 1 | 13.14** | 2.32 |
| 2. Aspartic acid | < 1 | 1.60 | 2.61 |
| 3. Homocysteine | 1.28 | 3.61* | 1.14 |
| 4. Cystine | < 1 | 5.00** | 1.21 |
| 5. Taurine | < 1 | 3.41* | < 1 |
| 6. Glutamic acid | < 1 | 1.30 | 3.84* |
| 7. Serine | 1.70 | < 1 | < 1 |
| 8. Glycine | < 1 | 4.97** | 1.21 |
| 9. Lysine | 1.73 | 6.17** | < 1 |
| 10. Arginine | < 1 | 8.85** | 4.86* |
| 11. Glutamine | < 1 | < 1 | 1.74 |
| 12. Alpha alanine | 1.60 | 6.36** | < 1 |
| 13. Beta alanine | < 1 | 6.90** | 1.55 |
| 14. Valine-norvaline | < 1 | 3.97* | 2.29 |
| 15. Isoleucine-leucine | < 1 | 4.04* | 1.24 |
| Five percent level of significance | 3.99 | 3.14 | 3.14 |
| One percent level of significance | 7.04 | 4.95 | 4.95 |

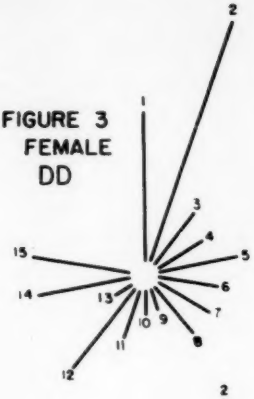
* Indicates significant at the 5% level.
** Indicates significant at the 1% level.

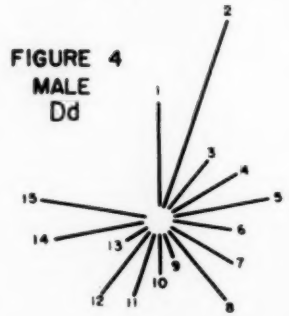
those drawn by WILLIAMS (1951). The length of each line in Figures 2 through 19 indicates the mean proportion of that amino acid in mice of that genotype, sex, and strain as taken from Tables 1, 2, and 3. The numbering is consistent throughout all figures as follows:

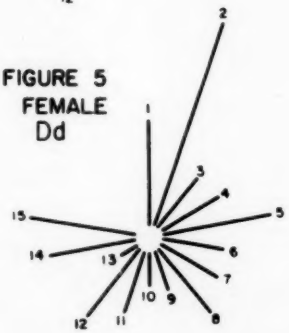
1. Glutathione
2. Aspartic acid
3. Homocysteine
4. Cystine
5. Taurine
6. Glutamic acid
7. Serine
8. Glycine
9. Lysine
10. Arginine
11. Glutamine
12. Alpha alanine
13. Beta alanine
14. Valine-Norvaline
15. Isoleucine-Leucine

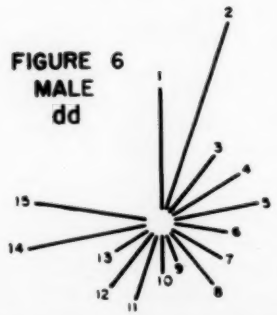
1. *Glutathione:* A significant F value was obtained for the between genotypes part of the two way analysis of variance for both the SEC/2 and the Furless strains. This leaves us with the inference that there is a difference between the genotypes within the SEC/2 strain, and within the Furless strain in the quantity of glutathione present in the blood. The analysis therefore, shows that the amount of the tripeptide glutathione in the blood is associated with the action of the genes responsible for the dense/dilute coat color in the SEC/2 strain, and with the genes responsible for the furless/normal coat in the Furless strain, or to other genes closely linked with these loci. Notice that the highest quantity of glutathione (Table 4a) is associated with the action of the homozygous dominant



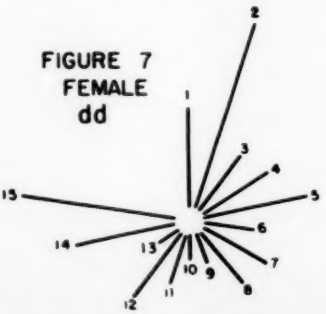
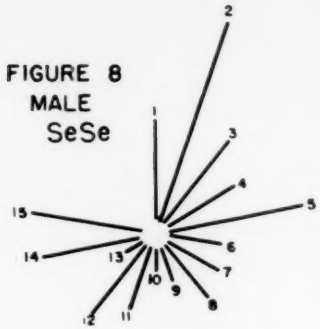
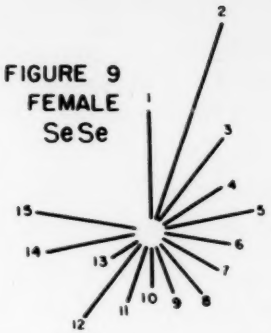
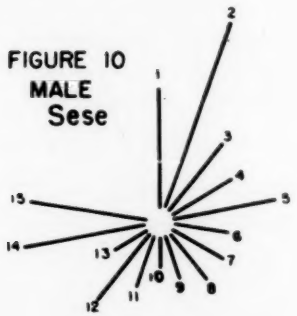
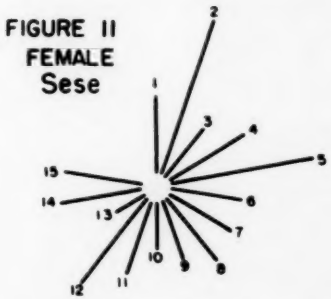



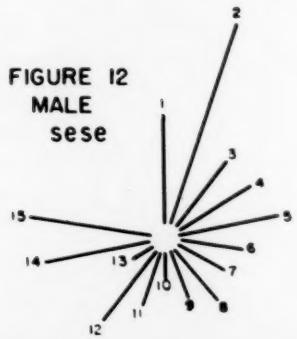

PLATE I. PATTERNS OF THE FREE AMINO ACIDS PRESENT IN THE BLOOD PLASMA OF MICE OF THE SEC/2 Gn-d STRAIN
(MEAN PROPORTION OF FOUR REPLICATES)



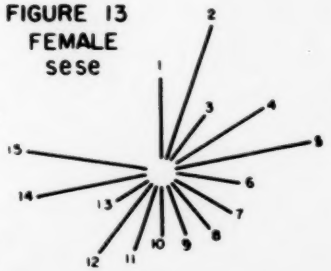
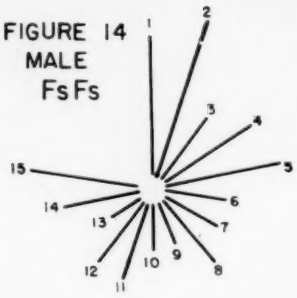
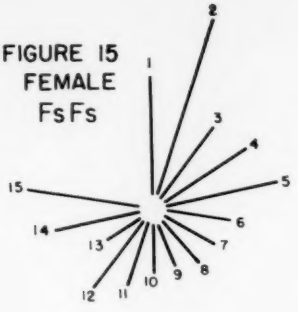
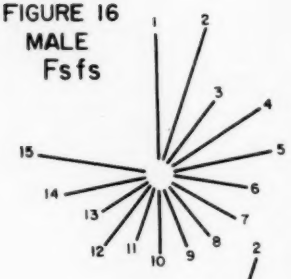
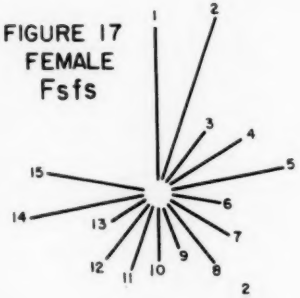



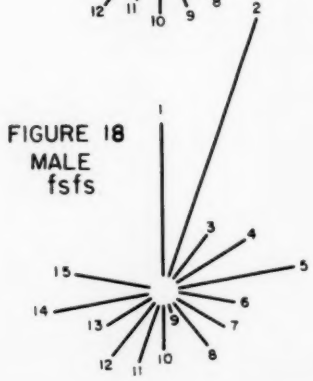

PLATE II. PATTERNS OF THE FREE AMINO ACIDS PRESENT IN THE BLOOD PLASMA OF MICE OF THE CBA/Ca-se STRAIN
(MEAN PROPORTION OF FOUR REPLICATES)



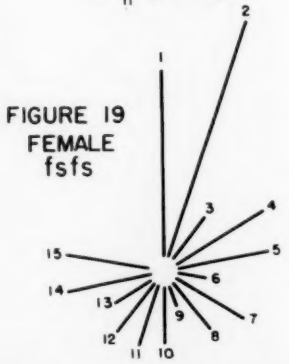




PLATE III. PATTERNS OF THE FREE AMINO ACIDS PRESENT IN THE BLOOD PLASMA OF MICE OF THE FURLESS STRAIN
(MEAN PROPORTION OF FOUR REPLICATES)

genotype in the SEC/2 strain. By contrast the homozygous recessive genotype shows the highest quantity of glutathione in the Furless strain.

No significant value is noted in the between sexes part of the analysis. No association can be claimed between sex and glutathione. An F value significant at the one percent level is noted between strains. That this is a real difference can be seen by inspection of the following table.

| | Total of Proportions |
|---|---|
| SEC/2 | 2212 |
| CBA | 1896 |
| Furl. | 2605 |

This shows that the proportion of glutathione in CBA mice is only about 19/26 of that in Furless mice (Figures 8 through 19). Therefore it may be proposed that the genetic backgrounds of the strains differ with respect to those genes associated with glutathione.

An F value significant at the five percent level was obtained for the interaction of sex and genotype in the SEC/2 strain. This indicates the effects of genotype and sex on amino acid content are not additive.

2. *Aspartic Acid:* There is a significant F value for the differences between genotypes in the Furless strain. This leads us to the conclusion that there is a difference in proportional content of aspartic acid between genotypes of the Furless strain. We would conclude further that the genes at the *Fs/fs* locus are associated with processes involving aspartic acid, with the homozygous recessive showing the greatest amount. Since there is no significant F value for sex or strain, there is no association indicated of aspartic acid with sex, nor with any difference in the genetic backgrounds of the strains.

3. *Homocysteine:* No evidence was found relating this amino acid to any of the genotypes segregating within any of the three strains tested. There is no evidence indicating any association between homocysteine and the sexes.

A difference in genetic background of the strains is indicated by the significant between-strains deviation in this amino acid. The proportion of homocysteine in the Furless strain averages only 13/16 of that in the CBA strain (Figures 8 to 19).

The absence of any significant F values due to interaction fails to show any additivity of the effects of genotype and sex, or sex and strain.

4. *Cystine:* There are no significant F values observed in between genotypes or between sexes analyses. There is, therefore, no evidence shown here for an association of cystine with either sex or with any of the genes segregating in these three strains.

A significant F value was obtained in the between-strains analysis. Inspection of the data in the following table shows that the Furless strain has the highest average proportion of cystine in its blood, the CBA strain next highest and the SEC/2 lowest with only about 7/9 the proportional amount present in Furless.

| | Total of Proportions |
|---|---|
| SEC/2 | 1437 |
| CBA | 1609 |
| Furl. | 1806 |

This difference, too, can be attributed to differences in the genetic backgrounds of the strains.

5. *Taurine:* The analysis indicates no relation of taurine to sex or to any of the genotypes tested. The difference between strains that is indicated by the F value (significant at the five percent level) is again attributable to genetic background differences. The CBA strain shows the highest proportion of taurine, and SEC/2 the lowest.

Two F values significant at the one percent level were obtained for interactions in the genotype by sex analyses. The subsequent rejection of the null hypothesis indicates the effects of genotype and sex on the amino acid content are not additive in the SEC/2 and CBA strains.

6. *Glutamic Acid:* There is no relationship shown between glutamic acid and genotype in any of the three strains tested.

A significant F value was obtained between the sexes in the Furless strain, male mice having a greater proportion than female mice. This indicates a possible association between glutamic acid and genes associated with the sex factors. This association was not carried over, however, when the mice of all three strains were grouped according to sex.

The analysis failed to demonstrate any difference among the strains in glutamic acid content, but the strains by sexes interaction exhibited a significant F, indicating the nonadditivity of their effects.

7. *Serine:* The analysis failed to show any significant difference between genotypes, sexes, or strains tested in the proportion of the amino acid serine. Therefore, I assume that the genetic backgrounds of the strains do not differ markedly with respect to the genes associated with serine. Notice the fairly constant length of the No. 7 line in Figures 2 through 19. There was also no interaction indicated between the main effects.

8. *Glycine:* The possible association of glycine with the dense/dilute genotype segregating in the SEC/2 strain is indicated by a significant F value in that strain analysis. In the following table we can see that the heterozygous genotype is associated with the highest average proportion of glycine in both the SEC/2 and the CBA strains, and very close to the highest in the Furless strain.

| Generalized Genotype | SEC/2 | CBA | Furl. |
|---|---|---|---|
| *AA* | 510 | 446 | 456 |
| *Aa* | 568 | 464 | 452 |
| *aa* | 465 | 424 | 388 |

The homozygous recessive is associated with the lowest average proportion in all three strains.

There can be no sexual association claimed on the basis of these analyses, but a strain difference is indicated by the F value, significant at the one percent level, obtained in the between-strains comparison. The average proportion of glycine in the blood of Furless individuals is only 5/6 of that in SEC/2 mice, as shown here.

| | Total of Proportions |
|---|---|
| SEC/2 | 1543 |
| CBA | 1334 |
| Furl. | 1296 |

9. *Lysine:* Significant F values are obtained for proportions of lysine associated with differences between genotypes within a strain, between sexes within one strain, and between strains. The difference between genotypes within the Furless strain is readily noticeable upon inspection of the following table. The homozygous recessive *fs/fs* shows a drastically lower proportion than either of the other two genotypes in the Furless strain, having only about 3/7 the proportional amount as does the heterozygote *Fs/fs* (Plate III, Figures 14 through 19).

| Generalized Genotype | SEC/2 | CBA | Furl. |
|---|---|---|---|
| *AA* | 221 | 303 | 316 |
| *Aa* | 266 | 364 | 361 |
| *aa* | 240 | 349 | 147 |

It is interesting to note that all of the heterozygotes exhibit a higher average proportion of lysine than do either of their corresponding homozygotes.

Genes on the sex chromosomes or those whose actions are influenced by sex may also be associated with the metabolic chemistry of lysine, as indicated by the difference between the sexes within the CBA strain. Although the female has a greater average proportion of lysine than the male in all strains tested, the magnitude of the difference in the SEC/2 and Furless strains is so small that the

| | SEC/2 | CBA | Furl. |
|---|---|---|---|
| Male | ↓ 351 | ↓ 455 | ↓ 410 |
| Female | (25) 376 | (106) 561 | (4) 414 |

number of degrees of freedom they add to the analysis more than compensates statistically for their contribution to the sex difference. These two strains may be more homozygous for those sex-associated genes affecting lysine than are the mice of the CBA strain.

A difference between strains is also indicated. The CBA strain mice have a strikingly higher average proportion of lysine in their blood than do either of the other strains tested (Figures 2 through 19).

10. *Arginine:* No association can be claimed between arginine and any of the three genotypes under test. A difference between the sexes (significant at the one percent level) in the SEC/2 strain was indicated in their content of arginine. The males had a higher proportion than the females. This difference does not persist when the mice of all three strains are grouped according to sex, since its effect is nullified by an opposite one in the CBA strain.

The strains differ in their average proportion of arginine. Mice of the Furless strain have a higher proportion of arginine than either of the other strains. This is true for each genotype in between-strains comparisons. A strains by sexes interaction is also indicated.

11. *Glutamine:* I failed to find any significant difference in proportion of glutamine relative to sex, genotype, or strain comparisons. I assume the genetic backgrounds of the three strains are similar for those genes influencing glutamine. Notice the fairly constant length of line No. 11 in Figures 2 through 19.

12. *Alpha Alanine:* The dense/dilute locus or other loci closely linked to it segregating in the SEC/2 strain are apparently involved in the control of the metabolic chemistry of alpha alanine. The homozygous dominant genotype has the greatest proportion, the homozygous recessive the least. The F value obtained for the among genotypes comparison is significant at the five percent level.

Although no relation to sex is found, an F value significant at the one percent level indicates a difference among strains. The proportion of alpha alanine in the Furless strain is only 8/10 of that in the CBA strain. Here, too, the genetic backgrounds of the three strains must differ with respect to those genes associated with the amino acid alpha alanine.

13. *Beta Alanine:* With no relation being shown to any of the segregating loci, or to sex, it can be assumed that the genes in the genetic background control the chemical processes involving beta alanine. The genetic backgrounds do seem to differ relative to the genes associated with this amino acid since an F value significant at the one percent level is obtained when comparing the strains. The average proportion of beta alanine in SEC/2 is but 7/10 of that in the Furless strain.

14. *Valine-Norvaline:* There is a possible association of valine and/or norvaline with the genes at the dilute locus segregating in the SEC/2 strain. The homozygous recessive *d/d* has the greatest proportion, while the other two genotypes are equal in their proportion.

An association with sex is indicated in the CBA strain by an F value significant at the one percent level. Most of the difference between the sexes appears to be due to interaction of sex and the heterozygous genotype. In CBA males the heterozygote has the *highest* amino acid level, while in heterozygous CBA females the level is suppressed. The proportion in the Furless strain females exceeded that in the Furless strain males to an extent great enough to nullify this difference when mice of all strains were grouped according to sex. The genetic background in the CBA strain may be such as to allow a sex difference to show.

The strains differ relative to the proportion of either valine or norvaline, or the combination of them *viz.* a total of proportions of 1891 in SEC/2, 1929 in

CBA, and 1693 in Furless. Interactions between genotype and sex were obtained for the SEC/2 and CBA strains.

15. *Isoleucine-Leucine:* A significant F value is obtained when comparing genotypes within the Furless strain, and when comparing strains. There is no evidence associating these amino acids with sex differences. The association of either one or both of these amino acids with the Furless locus (or other closely linked loci) segregating in the Furless strain is indicated by an F value significant at the five percent level. Here again the homozygous dominant has the greatest proportion and the homozygous recessive the least. The strain difference found here indicates differences in genetic background between the three strains for those genes associated with the biological processes involving either isoleucine, leucine, or both.

## SUMMARY

This research disclosed statistically significant differences between the genotypes in mice of the SEC/2 Gn-*d* strain for glutathione (*DD* high), glycine (*Dd* high), alpha alanine (*DD* high) and valine-norvaline (measured together) (*dd* high). No differences were found between the genotypes in the CBA/Ca-*se* strain. Between the genotypes segregating in the Furless strain, there were significant differences found with respect to glutathione (*fsfs* high), aspartic acid (*fsfs* high), lysine (*Fsfs* high), and isoleucine-leucine (measured together) (*FsFs* high). Significant differences in the level of arginine, lysine, valine-norvaline, and glutamic acid were found between the sexes within a particular strain. Male and female SEC/2 mice differed in the proportion of arginine in their blood the males being higher. CBA males differed from the females in their proportion of lysine (females higher than males), and valine-norvaline (males higher than females), while the proportion of glutamic acid in Furless males exceeded that in females by a statistically significant amount. All of these sexual differences disappeared, however, when all mice of all three strains were grouped according to sex and analyzed together. The strains differed statistically with respect to their relative proportions of glutathione, homocysteine, cystine, taurine, glycine, lysine, arginine, alpha alanine, beta alanine, valine-norvaline, and isoleucine-leucine. Despite the presence of the many significant differences, a fairly standardized metabolic pattern can be generated for each inbred strain of mice.

## ACKNOWLEDGMENT

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# MORPHOGENETIC STUDIES OF THE RABBIT. XXIII. THE EFFECTS OF THE DACHS GENE DA (CHONDRODYSTROPHY) UPON LINEAR AND LATERAL GROWTH OF THE SKELETON AS INFLUENCED IN TIME[1]

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INHERITED skeletal abnormalities which may be, and have been, classified fundamentally as the result of disproportionate reduction of localized parts manifest a wide range of species patterns with respect to the degree and location of effect upon the skull and limbs, and in some cases the vertebrae. When homozygous these abnormalities are lethal at a relatively early age in the most severely affected, such as the Creeper fowl, (LANDAUER 1932) achondroplastic rabbit (*ac*), (PEARCE and BROWN 1945), and certain of the cattle dwarfs. They vary considerably in their expression when heterozygous from almost none to obvious abnormality, whereas animals such as the Bassett hound and Dachshund (commonly considered disproportionate dwarfs) are able to live apparently normal lives though homozygous. Between these two groups is the dachs rabbit (SAWIN and CRARY 1957) which is neither a severe and early lethal, nor one which can be said to live out as a breed a relatively complete and normal life span. In some genomes the gene induces (when homozygous) a severe crippling, whereas in others it permits survival to adulthood and production of viable young, although few of the young so produced can be raised without a foster mother. Thus, considering the disproportionate dwarfs as a group, there is a wide range of variation, both with respect to time of onset of gene effect and to its effect upon the life span.

That time is an important factor in the expression of such anomalies was brought out by STOCKARD (1941) on dogs, and particularly by LANDAUER (1934) in early studies of the Creeper fowl. The latter repeatedly emphasized this fact again in connection with "Brachypodism" in mice (LANDAUER 1952).

Although the number of cases in which onset and duration of action are at all pinpointed in time is almost exclusively restricted to LANDAUER's work, our recent studies of the dachs gene (CRARY and SAWIN 1958) have demonstrated quantitative changes which in this case at least suggest a more extensive effect of the gene in time than has been demonstrated in any previously described case. The importance of differential species patterns of development has been summarized by LANDAUER (1952). These suggest the importance of study of dissimilarities of the normal and abnormal growth patterns in time. LANDAUER has

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particularly called attention to the fact that the differential response of anterior and posterior extremities in the case of the brachypod mouse is a function of the normal developmental pattern of higher vertebrates. We have been able to show what seems to be a consistent pattern of regional differentiation of vertebrae particular to the rabbit, which is varied by race (SAWIN and CRARY 1956) and also in time if comparison is made of a fetal (22 day) population with the adult (CRARY, SAWIN and ATKINSON 1958).

This paper reports effects of the dachs gene observed at 21 days. It confirms our previous observation at 22 days that the reduction apparent in the adult dachs is already present in the fetus. It further shows that differences in bone measurements between two populations taken during this 24-hour interval (21 and 22 days) provide a clue as to the time of onset of gene activity. These observations, compared with those taken in newborn young at 31 days (RAFFERTY 1956) and with those of the adult, determine in time and place the limits of the dachs gene activity which are critical to bone growth.

## MATERIALS AND METHODS

Sixty-one rabbit fetuses, classified by ear type (CRARY and SAWIN 1958) as ten dachs (*DaDa*), 34 heterozygotes (*Dada*), and 17 normals (*dada*) were taken by Caesarian section at 21 days postcopulation. As in our previous studies, they were fixed in aceto-formalin alcohol for 15 minutes, macerated in one percent KOH, stained with alizarin, and cleared in 70 percent glycerine and alcohol (equal parts). These fetuses were classified by ear type only, as none of the measurements taken segregated into three nonoverlapping groups.

Ninety-one measurements of length and width of skull, vertebrae, and limb bones were made using a binocular microscope. A 9× ocular and 3× objective were used for vertebrae and limb bones, a 1× objective for skull bones. Exceptions were certain humeri, too long to be seen in one field, and frontal and parietal bones. These were measured with compass and mm rule to the nearest half mm. After computation, means were converted to mm for skull and limb measurements and to tenths of mm for vertebrae, with standard deviations adjusted accordingly, so that they would be comparable with measurements at 22 days (CRARY, SAWIN and ATKINSON 1958). Lengths were taken along the longitudinal axis and widths at the anterior end with the exception of parietal, scapula, ilium, and ischium, which were measured at the point of greatest width perpendicular to the longitudinal axis. Significant differences between the three groups were determined by both F and t tests of variance. Incidence of centra and neural arches was noted and the percents of those present were graphed by genotype. Measurement of neural arches is not feasible because of irregularity of shape.

## RESULTS

*Body weight:* The mean weights of the fetuses of this study are shown in Table 1, together with the standard error, variability, and significance. In contrast to previous observations made at 22 days and at postlactation ages where between-

TABLE 1

*Mean fetal weights (gm), standard deviations (S.D.), variability (C.V.), and tests of variance in the three genotypes segregating from heterozygous parents* (Dada)

| | *DaDa* | *Dada* | *dada* |
|---|---|---|---|
| Number | 9 | 34 | 16 |
| Mean | 5.02 | 5.16 | 5.31 |
| S.D. | 0.39 | 0.70 | 0.53 |
| C.V. percent | 7.77 | 13.57 | 9.98 |
| | *DaDa vs. Dada* | *DaDa vs. dada* | *Dada vs. dada* |
| SE | 0.23 | 0.25 | 0.18 |
| t | 0.61 | 1.16 | 0.83 |
| | F = n.s. | | |

genotype differences are highly significant, at 21 days the differences of approximately five percent between dachs and normal and three percent between dachs and heterozygote are not statistically significant.

*Skull:* Examination of the percent difference in length and width measurements of the skull bones (Table 2) of homozygous dachs (*DaDa*) and normal (*dada*), column 5, shows that most of the differences are small (less than ten percent), and in a surprising number of cases measurement of the dachs is greater than normal.

Of the endochondral bones, only the basioccipital is significantly smaller ($p = <.01$). It is noticeably misshapen compared with both normal and heterozygote and by measurement is reduced more than 20 percent from normal in both length and width. The basisphenoid is not ossified in some individuals of all three genotypes and its size when present is quite variable. This probably accounts for lack of any significant difference. Presphenoid at this age is not ossified.

Of the membrane bones, the frontal bones of dachs show the greatest effect. They are reduced by 30 percent over normal for length ($p = <.01$) and by eight percent for width ($p = <.05$). Heterozygotes are slightly larger than normals.

Mandible depth of dachs is less by eight percent than either normal or heterozygote. The interparietal is the only other skull bone to show a significant effect at this age. However, it is not ossified in any dachs, and not in all normals or heterozygotes. In those heterozygotes which are ossified it is nearly ten percent wider than the normals ($p = <.01$).

The variability in measurements of these bones (CV, columns 2, 3, and 4, Table 2) is high as compared with that in either adults or 22-day fetuses. (See SAWIN and CRARY 1957; CRARY, SAWIN and ATKINSON 1958.) On eight of the 21 measurements (four lengths, four widths) the ossification centers of the dachs are larger than the normal. In ten cases (six lengths and four widths) the heterozygotes are larger than either homozygous group. Thus it appears that in the skull almost a third of the differences between the dachs and normal are positive rather than negative. The significant differences are few and highly localized in the basioccipital and adjacent bones.

TABLE 2

*Average measurements of skull bones in millimeters, their variability and significance*

| Measurement | *DaDa* | | | *Dada* | | | *dada* | | | Percent diff. *DaDa vs. dada* | Analysis of variance | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | x | $\sigma$ | CV | x | $\sigma$ | CV | x | $\sigma$ | CV | | 3 groups | *DaDa vs. dada* | *DaDa vs. Dada* | *Dada vs. dada* |
| Skull length | 13.50 | 1.47 | 10.89 | 13.84 | 1.13 | 8.16 | 13.79 | 1.40 | 10.15 | — 2.1 | .. | .. | .. | .. |
| Width Zygo Arch | 8.15 | 1.23 | 15.09 | 7.65 | 1.12 | 14.64 | 7.65 | 1.41 | 18.43 | + 6.5 | .. | .. | .. | .. |
| Width Aud Meatus | 8.45 | 0.89 | 10.53 | 7.78 | 1.09 | 14.01 | 8.29 | 1.27 | 15.32 | + 1.9 | .. | .. | .. | .. |
| Basioccipital length | 1.87 | 0.46 | 24.69 | 2.56 | 0.34 | 13.35 | 2.51 | 0.51 | 20.18 | —25.5 | ** | ** | ** | .. |
| Basioccipital width | 1.26 | 0.19 | 15.18 | 1.62 | 0.18 | 11.41 | 1.64 | 0.22 | 13.19 | —23.2 | ** | ** | ** | .. |
| Basisphenoid length | 0.68 | 0.18 | 27.14 | 0.63 | 0.17 | 27.17 | 0.62 | 0.15 | 24.92 | + 9.7 | .. | .. | .. | .. |
| Basisphenoid width | 0.84 | 0.20 | 23.93 | 0.85 | 0.29 | 33.73 | 0.81 | 0.19 | 22.97 | + 3.7 | .. | .. | .. | .. |
| Mandible length | 7.97 | 0.91 | 11.37 | 8.45 | 0.66 | 7.79 | 8.15 | 0.82 | 10.02 | — 2.2 | .. | .. | .. | .. |
| Mandible depth | 3.38 | 0.35 | 10.43 | 3.65 | 0.29 | 7.84 | 3.68 | 0.49 | 13.36 | — 8.2 | .. | * | * | .. |
| Nasal length | 2.82 | 0.37 | 13.05 | 3.02 | 0.48 | 15.80 | 2.97 | 0.48 | 16.31 | — 5.0 | .. | .. | .. | .. |
| Nasal width | 1.95 | 0.12 | 6.05 | 1.90 | 0.20 | 10.80 | 1.93 | 0.18 | 9.15 | + 1.0 | .. | .. | .. | .. |
| Frontal length | 4.30 | 0.35 | 8.14 | 6.32 | 0.46 | 7.28 | 6.15 | 0.39 | 6.34 | —30.1 | ** | ** | ** | .. |
| Frontal width | 2.70 | 0.35 | 12.96 | 2.97 | 0.24 | 8.08 | 2.94 | 0.17 | 5.78 | — 8.2 | * | * | ** | .. |
| Parietal length | 5.10 | 0.46 | 9.02 | 4.97 | 0.55 | 11.07 | 5.03 | 0.67 | 13.32 | + 1.4 | .. | .. | .. | .. |
| Parietal width | 4.90 | 0.52 | 10.61 | 5.22 | 0.50 | 9.58 | 5.35 | 0.77 | 14.39 | — 8.4 | .. | .. | .. | .. |
| Exoccipital length | 1.63 | 0.48 | 29.48 | 1.56 | 0.32 | 20.32 | 1.41 | 0.28 | 19.72 | +15.6 | .. | .. | .. | .. |
| Exoccipital width | 2.77 | 0.16 | 21.24 | 0.79 | 0.17 | 21.03 | 0.82 | 0.16 | 19.37 | — 6.1 | .. | .. | .. | .. |
| Supraoccipital length | 1.16 | 0.36 | 30.78 | 1.34 | 0.42 | 31.72 | 1.25 | 0.37 | 29.67 | — 7.2 | .. | .. | .. | .. |
| Supraoccipital width | 3.36 | 0.27 | 7.94 | 3.56 | 0.76 | 21.30 | 3.54 | 0.88 | 24.89 | — 5.1 | .. | .. | .. | .. |
| Interparietal length | ... | ... | ... | 0.59 | 0.26 | 44.24 | 0.76 | 0.26 | 34.29 | ... | .. | .. | .. | .. |
| Interparietal width | ... | ... | ... | 3.37 | 0.64 | 18.95 | 3.06 | 1.13 | 36.91 | ... | .. | .. | .. | ** |

*Vertebrae*

(a) *Incidence of ossification centers:* At 21 days, ossification of the vertebrae is well started but still incomplete. From 10–20 percent of individuals are lacking some ossification centers of the neural arches or centra, or both (Figures 1 and 2).

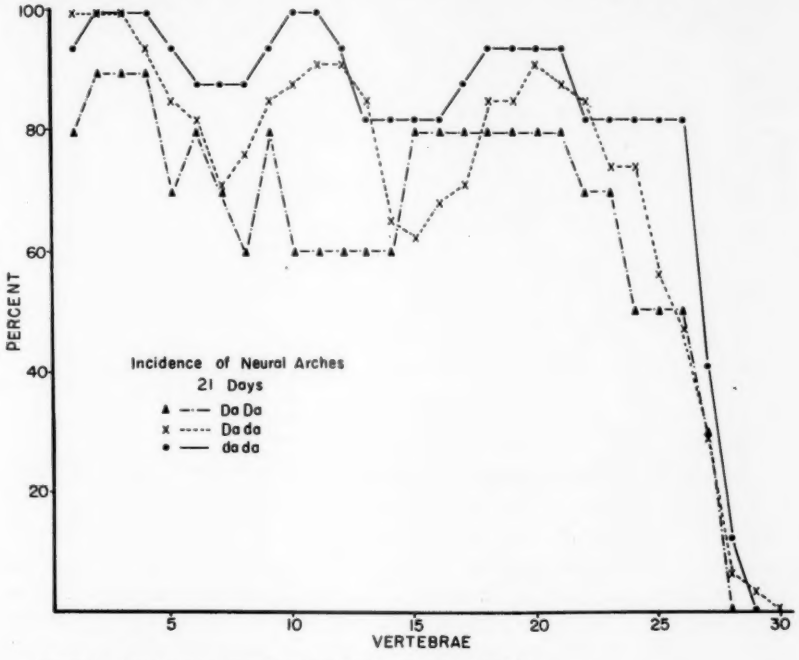


FIGURE 1.—Incidence of centers of ossification of neural arches.

This transitional state affords a ready qualitative means of assessing the relative effects of the dachs gene or any other genetic or environmental influence upon the vertebrae at this time. In this case, a comparison of Figures 1 and 2 with Figures 2A and 2B of SAWIN and CRARY (1956) shows that with respect to incidence of ossification centers of neural arches and centra all three genotype populations of the Dachs race are equal to or more advanced than the two other rabbit races III and X and in some respects they are advanced over IIIc, the most advanced of the races previously described. Incidence of ossification centers of centra of Dachs populations, normal, heterozygote, and homozygote, is more advanced in the thoracolumbar region than in IIIc, but more retarded in the cervical and thoracic regions. This should be kept in mind with reference to the quantitative observations described below. Further, it will be seen in Figures 1 and 2 that the retardation induced by the *DaDa* genotype is greater in neural

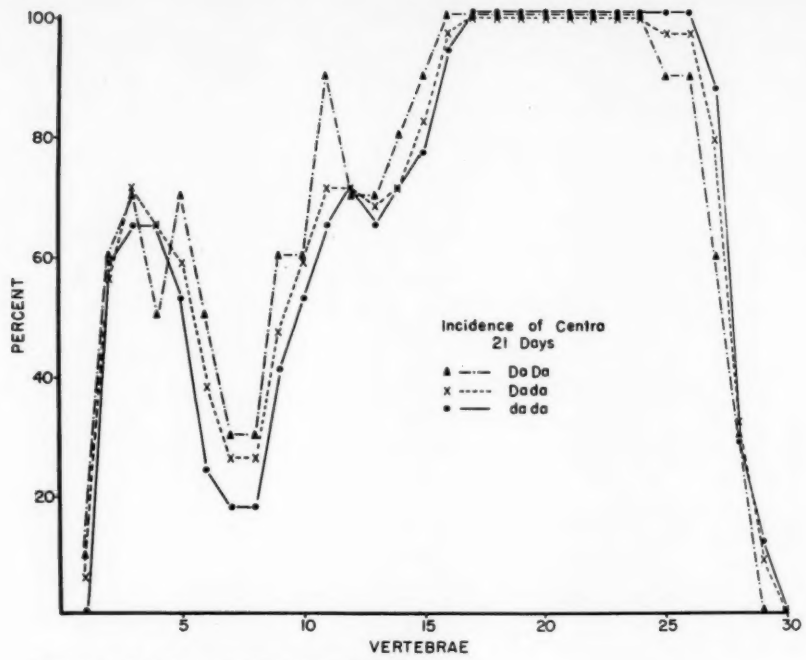


FIGURE 2.—Incidence of centers of ossification of centra.

arches than in centra. In fact, the dachs (*DaDa*) as a group show a higher incidence of centra ossified from vertebrae 5–11 than is found in either normals or heterozygotes, but posteriorly, at vertebrae 25–28 (sacral), the incidence is less, thus indicating a differential effect on neural arch *vs.* centra development and accelerated development anteriorly in the centra, whereas retardation is prevalent elsewhere.

(b) *Measurement:* The mean lengths and widths of ossified centra of the three genotypes at 21 days are shown in Figures 3 and 4 respectively. Here there are two striking observations. First is the relatively close equivalence of mean vertebral values of the dachs and normals. Actually, there are ten dachs vertebrae (1, 2, 4, 10, 12, and 15–19) which are longer than normal and three (1, 2, and 9) which are wider. Only three vertebrae (6–8) show the characteristic retardation in length observed in the dachs at later stages of development ($p = <.05$) and three vertebrae, 3 and 13 ($p = <.05$) and 6 ($p = <.01$) show retardation in width. All differences in which the dachs measurements are less than in normal genotype are in regions of relatively low incidence of ossification (compare with Figures 1 and 2). These are the same regions which in all rabbits tend to be relatively retarded. The *Da* gene thus tends to enhance normal retardation processes. The second observation is that nearly all heterozygous measurements

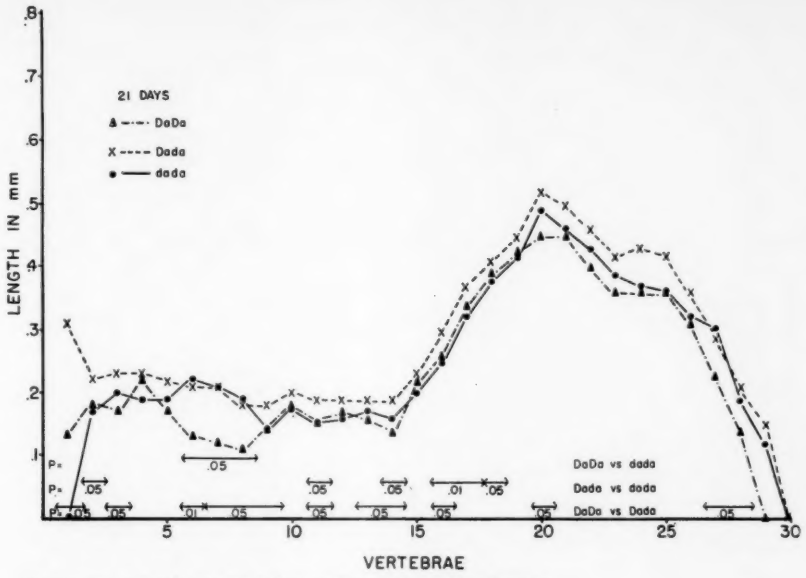


FIGURE 3.—Length of ossification centers of the vertebral centra.

(26 of the 29 for length, and 27 for width) are greater than normal. The significantly greater values for length are vertebrae 2–5 and a cluster (vertebrae 11, 14, and 16–18) centered around vertebra 15 (see Figure 1), and for width vertebrae 2 and 9. Since vertebral size of the heterozygote is in a general way large in relation to the normal, it is also relatively much larger with relation to the dachs. The most significant values in this case, as shown in Figure 1, tend to be centered anteriorly in the thoracic region (vertebra 6 for length and width), but they also extend more posteriorly. Length shows the largest number of significant differences.

Of further and particular interest is the fact that in the dachs (*DaDa*) the ossification observed on vertebra one, which is actually the ventral arch, there being no centrum in the atlas, is more advanced (both length and width) than in the normal, and is greater in the heterozygote than in either homozygote, whereas all vertebrae posterior to vertebra 20 are less than normal. This difference is greatest in vertebra 21.

Thus, as in the skull, the significant effects of the dachs gene on the vertebrae at this age are relatively small and highly localized, particularly in the homozygote. Likewise, as in the skull, variability is high and almost as often differences are positive as negative. Further, and most interesting, is the fact that so many heterozygous measurements are greater than those of either homozygote.

The significant differences are clustered anteriorly in both length and width, whereas at 22 days a larger proportion were found more posteriorly.

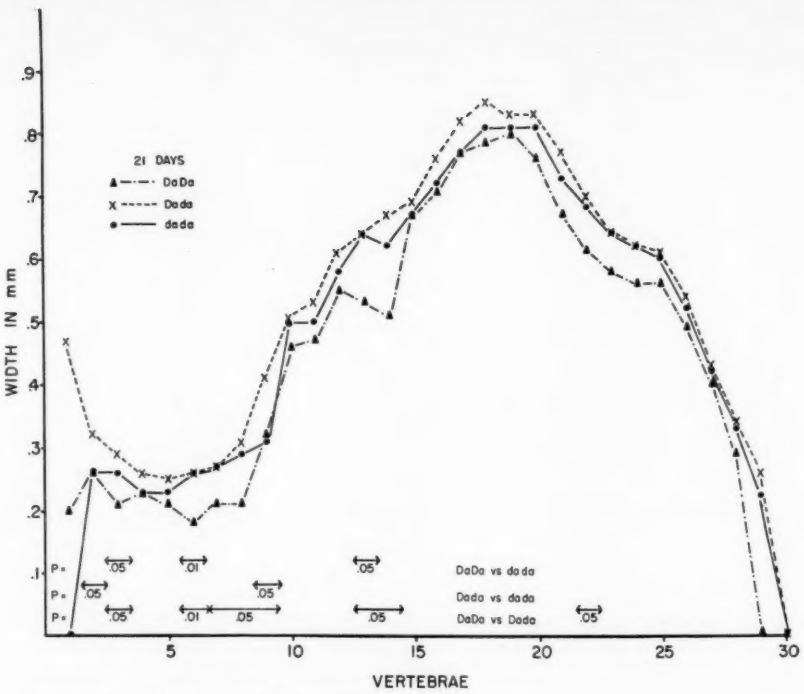


FIGURE 4.—Width of ossification centers of the vertebral centra.

*Limbs:* As in the skull and vertebrae, some of the skeletal units are significantly affected by the *Da* gene but others are not. In general, the proportion of limb measurements affected is greater than in skull and vertebrae and there is a high degree of localization. The significant length differences between dachs and the other two genotypes are confined to the girdles and the distal limb bones (ulna, tibia, and fibula) (Tables 3 and 4), whereas the intervening humerus and femur are relatively unaffected. As indicated by the relative or percent reductions (column 5) and significance of differences (column 6) the effect seems to be more proximal in the fore limb than in the hind limb. Both clavicle and scapula are affected ($p = <.01$) but of the distal bones, only the ulna. In the hind limbs the ischium, which is present in only a very few of the dachs at this age, and the femur are unaffected, but both tibia and fibula are significantly affected ($p = <.01$). A similar situation is found for width, but the pattern of localization is not the same. In the fore limb scapula and humerus are significantly reduced by the *Da* gene (77% and 20% respectively) whereas clavicle, radius, and ulna are not. The pattern is thus the reverse of that of length. It is also noteworthy that the heterozygous ulna tends to be wider than either homo-

TABLE 3

*Average length measurements of the limb (mm)†*

| Measurement | *DaDa* x | *DaDa* σ | *DaDa* CV | *Dada* x | *Dada* σ | *Dada* CV | *dada* x | *dada* σ | *dada* CV | Percent diff. *DaDa vs. dada* | Analysis of variance: 3 groups | *DaDa vs. dada* | *DaDa vs. Dada* | *Dada vs. dada* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Clavicle | 1.93 | 0.40 | 20.71 | 2.20 | 0.33 | 15.05 | 2.33 | 0.30 | 12.94 | −17.2 | * | ** | * | .. |
| Scapula | 1.43 | 0.50 | 45.41 | 2.52 | 0.29 | 11.68 | 2.61 | 0.30 | 11.48 | −45.3 | ** | ** | ** | .. |
| Humerus | 3.24 | 0.25 | 7.64 | 3.35 | 0.35 | 10.42 | 3.29 | 0.39 | 12.01 | − 1.27 | .. | .. | .. | .. |
| Radius | 1.98 | 0.30 | 15.04 | 2.40 | 0.26 | 10.71 | 2.29 | 0.35 | 15.15 | −13.5 | .. | .. | * | .. |
| Ulna | 1.80 | 0.22 | 12.31 | 2.58 | 0.35 | 13.62 | 2.49 | 0.34 | 13.85 | −27.8 | ** | ** | ** | .. |
| Ilium | 1.15 | 0.19 | 16.42 | 1.26 | 0.20 | 15.77 | 1.37 | 0.16 | 11.67 | −15.6 | * | ** | .. | .. |
| Ischium | 0.47 | 0.18 | 37.19 | 0.53 | 0.19 | 35.77 | 0.54 | 0.16 | 30.21 | −11.4 | .. | .. | .. | .. |
| Femur | 2.23 | 0.31 | 13.78 | 2.42 | 0.26 | 10.67 | 2.32 | 0.28 | 12.19 | − 3.5 | .. | .. | .. | .. |
| Tibia | 2.40 | 0.31 | 13.01 | 2.84 | 0.34 | 12.15 | 2.83 | 0.44 | 15.43 | −15.2 | ** | ** | ** | .. |
| Fibula | 1.45 | 0.35 | 24.17 | 1.91 | 0.36 | 18.78 | 2.10 | 0.39 | 18.81 | −30.6 | ** | ** | ** | .. |

† Calculations are based on measurements made in .1 mm converted to mm and carried to two places for tabulation.

TABLE 4

*Average width measurements of the limb (mm)†*

| Measurement | *DaDa* x | *DaDa* σ | *DaDa* CV | *Dada* x | *Dada* σ | *Dada* CV | *dada* x | *dada* σ | *dada* CV | Percent diff. *DaDa vs. dada* | Analysis of variance: 3 groups | *DaDa vs. dada* | *DaDa vs. Dada* | *Dada vs. dada* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Clavicle | 0.19 | 0.03 | 18.21 | 0.19 | 0.07 | 39.02 | 0.20 | 0.06 | 31.47 | − 5.0 | .. | .. | .. | .. |
| Scapula | 0.64 | 0.30 | 46.53 | 2.75 | 0.20 | 7.30 | 2.83 | 0.20 | 7.00 | −77.4 | ** | ** | ** | .. |
| Humerus | 0.91 | 0.08 | 9.25 | 1.12 | 0.16 | 13.98 | 1.13 | 0.18 | 15.72 | −19.5 | ** | ** | ** | .. |
| Radius | 0.54 | 0.13 | 23.13 | 0.55 | 0.07 | 11.91 | 0.56 | 0.08 | 13.70 | − 3.6 | .. | .. | .. | .. |
| Ulna | 0.69 | 0.08 | 12.08 | 0.75 | 0.08 | 10.73 | 0.69 | 0.10 | 14.65 | + 0.4* | * | .. | .. | * |
| Ilium | 1.27 | 0.12 | 9.76 | 1.50 | 0.13 | 9.02 | 1.43 | 0.09 | 6.56 | +11.3 | ** | ** | ** | .. |
| Ischium | 0.62 | 0.16 | 26.76 | 0.65 | 0.18 | 27.24 | 0.62 | 0.21 | 34.30 | − 0.6 | .. | .. | .. | .. |
| Femur | 0.70 | 0.08 | 11.45 | 0.81 | 0.07 | 8.74 | 0.80 | 0.09 | 11.45 | −12.5 | ** | ** | ** | .. |
| Tibia | 0.71 | 0.06 | 9.11 | 0.86 | 0.10 | 12.04 | 0.85 | 0.13 | 15.82 | −16.2 | ** | ** | ** | .. |
| Fibula | 0.36 | 0.10 | 27.48 | 0.37 | 0.05 | 13.46 | 0.36 | 0.08 | 21.29 | − 1.4* | .. | .. | .. | .. |

† Calculations are based on measurements made in .1 mm converted to mm and carried to two places for tabulation.

zygote. In the hind limb, the ilium, femur, and tibia are reduced in width in the dachs, but the proximal ischium and distal fibula are little affected.

When compared with the 22-day stage (CRARY, SAWIN and ATKINSON 1958), it appears that the patterns in both length and width are closely similar, but as in skull and vertebrae, the 21-day development is less generalized and because of this the localized reductions are more pronounced.

## DISCUSSION

The pattern of the statistically significant reductions of the endochondral bones of the skull, vertebrae, and limb measurements induced by the *Da* gene is closely similar to that found in the 22-day fetuses previously reported. It differs, however, in two conspicuous ways. *First*, throughout the skeleton and particularly in the vertebrae there are fewer significant reductions, and many of the variations are in a positive rather than negative direction. This, together with the fact that fetal weight of the dachs is not significantly reduced as compared with normal homozygotes, indicates a relative absence of the generalized effects of the gene at this time. It renders the localized pattern all the more apparent. Either the retardation effects of the gene have been slight up to this age and become manifest first in the localized areas, or if any substantial effect has been induced earlier it has been corrected by some sort of cyclic acceleration of localized parts of which as yet we know nothing. Until further information is available it seems logical to conclude that 21 days is close to the time of the initial effect of the *Da* gene. Considering that the life span of the homozygous dachs is nearly normal, as compared with the early lethality of other disproportionate dwarfs, at least one of which (Creeper) is known to be initiated as early as the first several days of development, late initiation of dachs effect is not surprising.

The *second* major difference in the reduction pattern induced by the *Da* gene at this age as compared with that of 22 days is in the relative position of the statistically significant localized reductions. Comparison has already been made of the differences in pattern between 22-day fetuses and the adult. We are now able to consider the 21 day fetus and, by reference to the work of RAFFERTY (1956), the newborn fetus of 31 days.

Reduction in the adult skull is centered on the basisphenoid and basioccipital (endochondral) bones, with membrane bones, except for the nasal, relatively unaffected. At 31 days and at 22 days reduction is more generalized, with localized effects more specifically on the occipital complex and the frontals. At 21 days, the generalized effects are not yet developed and the basioccipital and frontal are the significantly reduced skull bones. Thus, there appears to be a tendency for the reduction effects to be shifted slightly anteriorly with age in endochondral bones and secondarily in membrane bones.

As shown in Figure 3 the significant reductions for vertebral length in the dachs at 21 days are in vertebrae 6–8. At 22 days (CRARY and SAWIN 1958) they are in two clusters, one on cervical vertebrae 3, 5, and 6; the second on thoracic and lumbar vertebrae 13–28 (with two exceptions, 14 and 18) a situation which

is approximately the same at birth (31 days). In the adult, however, the more anterior cluster of significant differences has enlarged by involving additional vertebrae posteriorly and the 13–28 cluster is reduced by five vertebrae, none involving 18–28. Thus the same sort of groups of significant reductions of vertebrae have tended to persist into the adult, although there has been readjustment in growth and development in such a manner as to involve slight different member vertebrae in the clusters. The nature of this readjustment has tended to shift both clusters more posteriorly in their relative anteroposterior levels.

A similar sort of pattern change occurs with respect to width of the developing centra. One cluster of centra showing significant differences is initiated in the thoracolumbar region at 21 days with vertebra 13. At 22 and 31 days this cluster is considerably increased to involve vertebrae 15–25 (thoracolumbar in position). In the adult it becomes centered on vertebra 25. A second significant cluster of vertebrae showing retardations in width is initiated anteriorly (vertebrae 3 and 6) at 21 days, is about the same at 22 days (vertebrae 2–7), and disappears completely by birth. It is also missing in the adult population.

In the limbs of the dachs there are similar significant changes in what for convenience may be referred to as a two-group pattern, one group of reductions tending to be initially centered proximally and the other distally. These are portrayed diagrammatically in Figure 5 with the percent reductions of length and width indicated for each unit and underlined where significant. In the fore limbs, lengths of clavicle and scapula at 21 days are reduced 17 percent and 45 percent respectively, and ulna 28 percent. Humerus (one percent) is unaffected. By 22 days all but clavicle are significant. (The same is true at 31 days, including metacarpals, which now are ossified). In the adult all lengths are significantly reduced. Thus the pattern of reduction established in the 21-day fetus is elaborated upon and with the exception of clavicle is perpetuated into the adult. The fact that clavicle reduction declines and is absent at birth, but reappears in the adult, may not be of significance in view of its relatively small size. The pattern for width is quite different. Only scapula and humerus show significant reductions at 21 days, to which is added radius at 22 days; ulna, but not radius, at 31 days. What is most interesting is that in the adult there are no significant reductions except in scapula. In other words, normal widths have been recovered in all but the one case. Similar sorts of pattern can be seen with respect to the hind limb, those of length tending for the most part to persist; those for width to flourish up to birth and then to decline, so that in the adult the significant retardations are in the ilium and femur. The effect in the latter seems obviously secondary and compensatory (Sawin and Crary 1957). It is also particularly significant that the pronounced retardations have occurred relatively early and quickly, around 21 and 22 days, and that the greatest reductions in both length and width later become less pronounced around the time of birth. In the limbs the pattern of change with age appears more complex than in the vertebrae, but certain specific generalizations can be made.

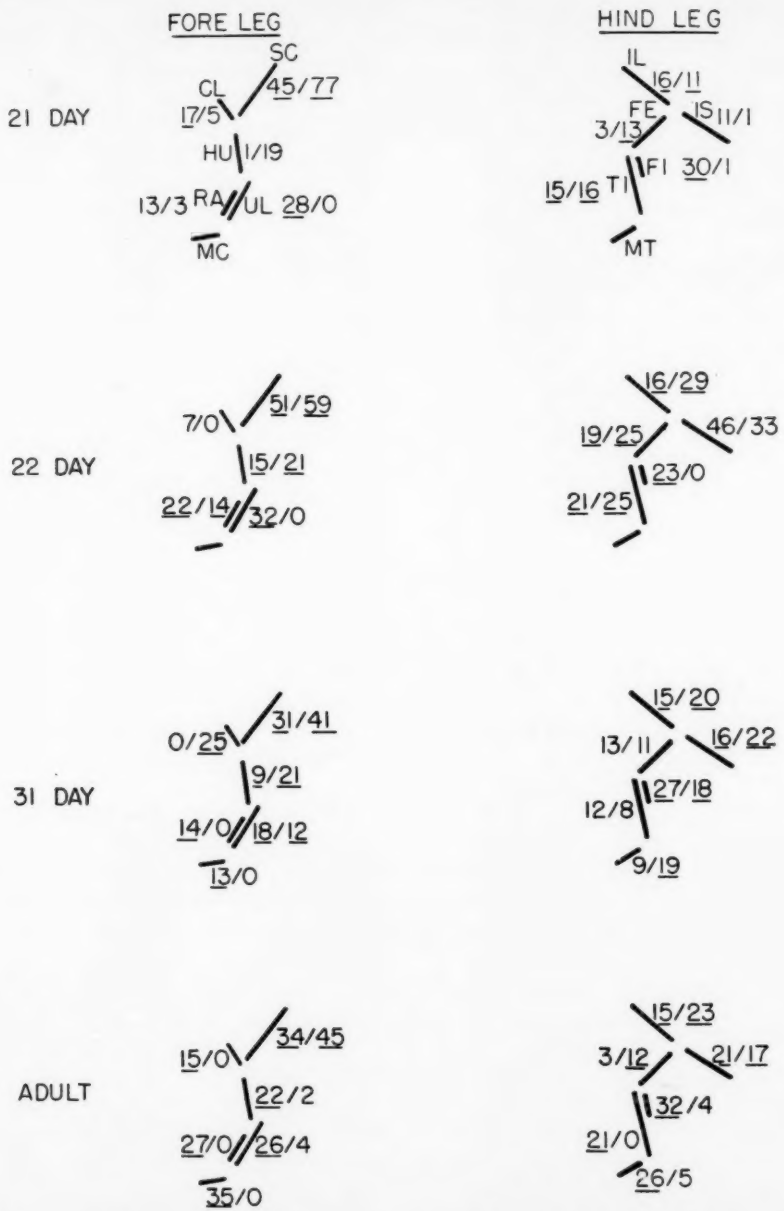


FIGURE 5.—Percent reduction of the fore and hind limb bones affected by the dachs gene and their significance at the several ages. Length is given first, followed by width. Statistically significant reductions ($P = <.01$) are underlined. Bones are indicated by their first two letters. Metacarpals and metatarsals are not ossified in 21 and 22 day fetuses.

These changes in the relative pattern of manifestation of reduction effect of the dachs gene fit the general conclusion of LANDAUER, "that such genes act upon certain fundamental dissimilarities of normal growth patterns which amount to the preservation of infantile or fetal proportions," to the extent that the retardation in length of the long bones, manifest as early as 21 days postcopulation, does seem to be perpetuated and enhanced in the adults. They do not compare, however, with respect to width which is determined by periosteal bone growth, and apparently does not come under quite the same reduction influences. Reduction of longitudinal bone growth is a characteristic typical of disproportionate dwarfs and is usually attributed to a defect in endochondral development. Reduction of lateral growth is not considered typical, but does occur in some cases as in the Dachs. It may be a reduction of the periosteal growth, but as such it is unlikely that it is the same process as that affecting longitudinal reduction since it is not correlated in point of time. In those cases in other species where width of bone is increased it has been regarded as a secondary or compensatory effect, evidence for which is also present in the skull and femur of the dachs as already suggested (SAWIN and CRARY 1957). LANDAUER (1952) interpreted the differential patterns of the extremities of the Creeper fowl, Ancon sheep, and brachypod mouse as occurring on the basis of a "pattern of development peculiar to higher vertebrates, the nature of which is unknown." In those cases, the major differential is that between fore and hind limb, the latter being the most reduced. The same seems to be the case with the human disproportionate dwarf (KNÖTZKE 1929) and with "phocomelia" of the mouse (GLUECKSÖHN-WAELSCH *et al.* 1956). With the dachs rabbit it is significant that the greater quantitative effect is in the fore limb. This observation tends to minimize the significance of a generalized vertebrate pattern and directs attention to the species pattern. Each of the species disproportionate dwarfs has a slightly different pattern in the limbs. In the Creeper fowl there is a proximo-distal gradient of increased reduction, whereas in the brachypod mouse, dachs rabbit, and Ancon sheep the differential effects concern different localities of the limb, and may be localized at one or two points. In the brachypod and dachs mutations the effect is greater proximally and (to a lesser extent) distally in the terminal segments. In the Ancon they are greater distally (LANDAUER and CHANG 1949). Similar *localized differentials* have been noted in vertebrae as well as limbs in other species, although they are not as prominent as in the limbs. CHANG (1949) showed evidence of them in the lumbar vertebrae of the Ancon, where they are accompanied by scoliosis and fusion, and, as in the dachs, also in vertebrae 1 and 2, although less pronounced. They are also reported by KNÖTZKE (1929) in the lumbar region of man, which is broader than normal.

CHANG also observed localized differentials in the Ancon skull; STOCKARD (1941) found they constitute a very prominent part of the skull pattern of the dachshund as compared with other breeds, and JULIAN *et al.* (1957) observed them in the "short headed" variety of disproportionate dwarfs in cattle. In the latter case, premature fusion of the spheno-occipital synchondrosis is a typical

accompaniment, and this is not uncommon in the dachs. The complexity of morphological relations in the skull are three dimensional and therefore more complex and more difficult to analyze in a manner which will identify or define the basic dimensional process involved.

Localized differential patterns are not confined to disproportionate dwarfism, however. PYLE and SONTAG (1943); FRANCIS, WERLE, and BEHM (1939); and CRARY and SAWIN (1949) have shown that they occur in the normal patterns in time of onset of ossification of the epiphyses of endochondral bones. In these patterns, two features have been conspicuous to several investigators. *First* is the fact that although relative age at onset of ossification of each bone may vary in relation to that of its neighbors, the order or pattern in which they appear tends to be the same for each species; *second*, the order of union of epiphyses with the diaphyses tends to be the reverse of the order in which they first appear. Thus there appears to be a curious interrelationship between the species pattern and time. That the pattern actually changes in time and is under physiological influences of external origin has been noted by MARTIN (1928), and SCAMMON and CALKINS (1929) in man and by LANDAUER (1952) in the chicken. In man the ulna-humeral indices rise during the fetal period, and decline between birth and maturity, whereas in the chick the pattern is reversed, declining prior to hatching and increasing thereafter. It appears that the general nature of the pattern changes of the disproportionate dwarf genes tends to be similar, whereas the specific patterns of the changes induced by the several genes are almost as varied as are other differences between the species in which they arise. Whereas LANDAUER (1952) has contended that such differential patterns of development of disproportionate dwarfs are functions of the normal development of higher vertebrates, from the above comparisons we are inclined to the belief that they occur as a result of the interaction with more specific patterns of development. Many of the differences in disproportionate patterns may be associated with species or race differences in pattern of development and some of the variations could possibly be associated with specific modifying genes. In view of (1) the close similarity in the syndrome of effects of so many disproportionate dwarf mutants, (2) their wide distribution among a number of species of domestic animals, (3) the numbers of them occurring in several species, and (4) the nature of the differences between them which essentially seem somehow closely related in time, it seems altogether likely that some of the differences are basically a function of the interaction with the genome in which the mutant appears to have occurred, or perhaps the time at which the gene begins to function. Whether, as LANDAUER also concluded, the gene action can be in all cases directly associated with those parts whose growth rate is greatest at the time the gene action is initiated is not clear. It would seem that in the dachs the shift in significant values from one anteroposterior level to another in successive age groups, occurring as it does in the vertebrae, skull, and to a lesser degree in the limbs, would imply action over a longer period of time and even perhaps a specificity of action in time as affecting the two types of bone development.

The later masking changes noted in the dachs, particularly in width, have not been observed heretofore in the cases of the other disproportionate growth genes and this could well be due to the lack of critical material, either in those cases in which homozygotes are characteristically lethal or because the value of more extensive examination longitudinally in time, particularly in the axial skeleton, has not been recognized and carried out.

Transposition of the reduction effect of a specific gene as modified in time which by its action is here demonstrated to mask some of its earlier effects offers a possible mechanism of gene activity which, if investigated further, may account for some of the sporadic and congenital variations of the morphologist and aid in explaining some of the threshold or quasicontinuous variations of the geneticist.

The fact that so many genes inducing disproportionate growth display the same sort of generalized pattern and primarily seem to differ only in the relatively greater expression of certain different localized portions of that pattern indicates some common element in the processes necessary to their fulfillment. The special pattern of growth characteristics induced by the *Da* gene, which seems in marked contrast to the early lethality of the Creeper fowl and some other disproportionate dwarfs, together with those of the recessive lethal achondroplasia *ac*, primordial dwarfism (nanosoma vera) *Dw*, and with the specific racial differences in growth pattern now available in the rabbit (Sawin nd Crary 1956) would seem to offer an unusual opportunity for analysis of possible genetic agencies bringing about changes in relative size.

## SUMMARY AND CONCLUSIONS

This study concerns the body weight and skeletal measurements of 61 rabbit fetuses of age 21 days postcopulation. They were derived from parents which were heterozygous for the dominant gene *Da* which induces a chondrodystrophic type of disproportionate growth and hence fall into three distinct genotypes identified by ear type. Four of the seven mothers had contributed to similar populations of 22 day fetuses previously described. The homozygous dachs population, *DaDa*, at 21 days shows a remarkable absence of the generalized reduction of both body size and skeletal measurements, particularly length of centra, observed at 22 days. This difference, together with the relatively high incidence of ossified vertebral centra for this age (compared with the other genotypes) seems to indicate that 21 days is in close proximity to the time of initial effects of this gene. It also brings into relief the closely similar pattern of localized reduction effects of the gene to that recorded previously for 22-day fetuses and adults. Its deviations at this age away from the adult pattern, together with the shifts in anteroposterior levels of significant reductions in the adult which tend to mask certain of the fetal characteristics, are discussed in relation to the patterns of disproportionate growth by other genes in other species, the whole of which points to the tentative conclusion that the dachs is a gene whose effects are initi-

ated relatively late in fetal life, but once induced linearly they continue well toward maturity; whereas laterally they become masked at some time in postnatal development.

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# PREFERENTIAL SEGREGATION OF STRUCTURALLY MODIFIED CHROMOSOMES IN MAIZE[1]

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IN *Zea mays* there are two different types of chromosome 10, distinguishable not only on the basis of their morphology but also by the fact that one segregates preferentially at megasporogenesis and the other does not. The present investigation is concerned with the behavior of two modified chromosomes derived from an abnormal 10 which exhibits the phenomenon of preferential segregation.

LONGLEY (1937, 1938), working with strains of *Zea mays* from southwestern United States and Mexico, found an exceptional form of chromosome 10, known as the abnormal 10. According to RHOADES (1942, 1952), this chromosome differs from the normal 10 in the chromomere pattern of the distal region of the long arm and by the presence of an extra segment attached to the distal end of the long arm. The extra segment of chromatin consists of (1) a proximal euchromatic region, (2) a large heterochromatic segment, and (3) a distal euchromatic region. Figures 1–3 illustrate pachytene configurations of the knobbed and knobless homologues. It will be seen in Figures 1 and 2 that the abnormal 10 possesses three prominent chromomeres in the distal one sixth of the long arm which are not present in the normal strain (Figure 3). It should be also noticed that there is another less conspicuous chromomere near the base of the knob (Figure 2). The normal chromosome is shorter in length than the abnormal 10. In plants heterozygous for normal and abnormal 10, pairing of the normal strand ends in the region between the most distal of the three prominent chromomeres and the chromomere close to the knob.

In 1942 RHOADES discovered that this abnormal chromosome 10 segregated preferentially at megasporogenesis in plants heterozygous for a normal and an abnormal 10. He found, among the progeny of backcrosses in which the abnormal 10 carried the *r* locus (one unit from knob) and normal 10 the *R* locus, approximately 70 percent of the abnormal type (*r*) instead of the expected 50 percent. Evidence was also obtained by RHOADES (1952) that preferential segregation of the knobbed chromosome occurred only when the two homologues were heterozygous for the knob. In plants homozygous for the abnormal 10 and heterozygous for the marker gene *R*, he found random segregation of the knobbed chromosomes 10. From these tests RHOADES concluded that the causative factor or factors for preferential segregation are located either in the region of the

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dissimilar chromomeres or in the knob of the abnormal chromosome 10. Another possibility is that the euchromatic portion of the extra chromatin in abnormal 10 might be responsible for preferential segregation.

In this connection RHOADES' and VILKOMERSON's (1942) cytological studies of abnormal 10 are of importance. They found that abnormal 10 was responsible for the formation of neocentromeres (or secondary sites of centric activity) in chromosomes other than abnormal 10 when one of the two chromatids of a non-homologous dyad had a knob. More important still, the chromosome segments possessing neocentromeres move precociously to the poles. As a consequence, chromatids possessing neocentromeres reach the poles before those without. Neocentromeres occurred in plants both heterozygous and homozygous for the abnormal 10, but the extent of neocentric activity was less in heterozygous plants.

In 1952 RHOADES proposed the following scheme to account for the origin of neocentromeres: (1) in the presence of abnormal 10, the true centric regions yield a surplus of chromosomal fiber substance which is extruded from the centric regions of abnormal 10 and other non-homologous chromosomes and flows along the chromosomes until it comes in contact with a knob; and (2) in the presence of a knob this excess centric substance forms neocentromeres from which supernumerary fibers arise. According to RHOADES the knobs either stimulate neocentric activity or have a special affinity for this substance.

In view of the evidence that the abnormal chromosome 10 causes the formation of neocentromeres and concurrently produces the phenomenon of preferential segregation, it has been postulated by RHOADES (1952, 1955a) that the two are intimately related. He suggests that two events are essential for the occurrence of preferential segregation namely, crossing over and neocentric activity.

As a consequence of crossing over between the centromere and the knob in plants heterozygous for abnormal 10, heteromorphic dyads are formed, of which one chromatid is knobbed and the other is knobless. At anaphase I the knobbed

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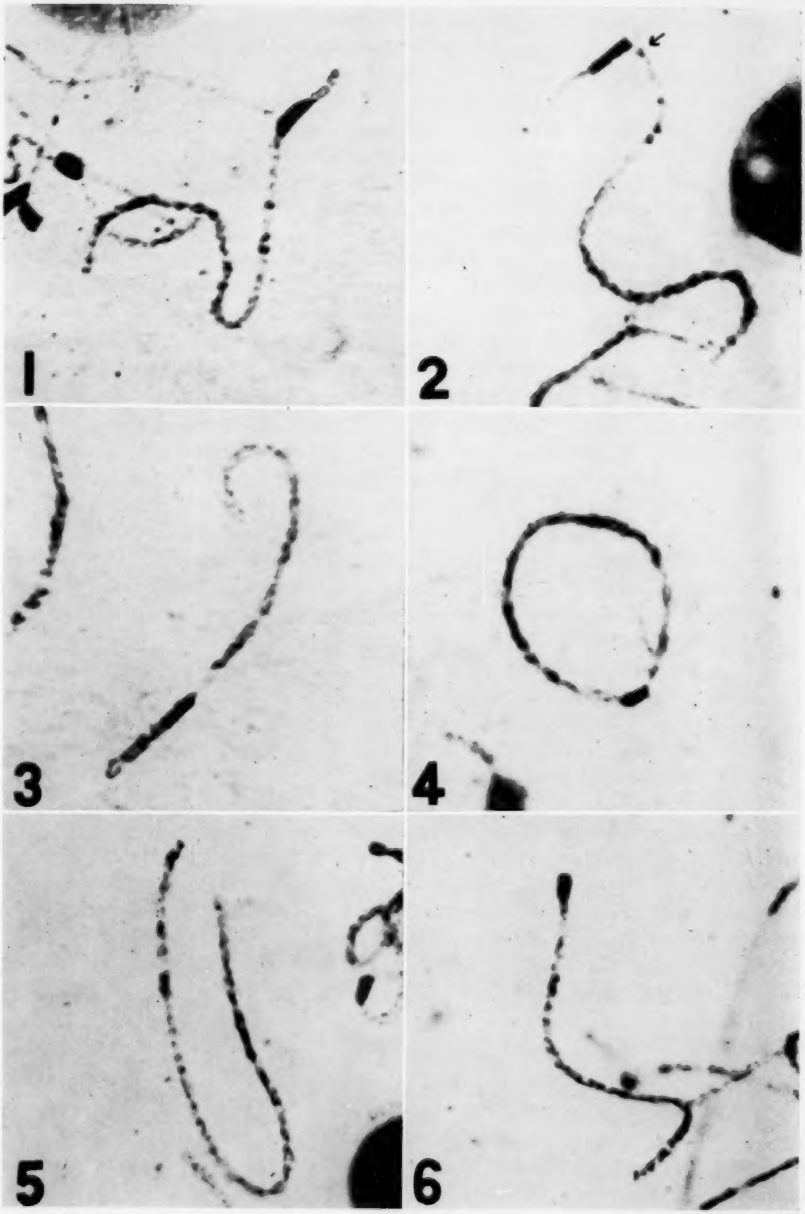
FIGURES 1–6.—Represent different forms of chromosome 10 at pachynema.

FIGURE 1 is the uncommon type of chromosome 10, known as abnormal 10. This figure, which was published in RHOADES (1952), is homozygous for abnormal 10. Note the three prominent chromomeres in the distal one sixth of the long arm, the large knob, and the euchromatic strand distal to the knob. FIGURE 2 shows a normal knobless 10 paired with the abnormal 10. The last chromomere (indicated by an arrow) is included in both of the altered chromosomes which are shown below. FIGURE 3 is the common type of chromosome 10, known as normal or knobless 10. Note that the three prominent chromomeres of the abnormal 10, as well as the knob and distal euchromatic thread, are not found in the knobless strain. This figure was obtained from RHOADES (1954). FIGURE 4 shows a ring chromosome for abnormal 10. This ring lacks a small portion of the short arm, approximately one half of the knob, and the entire strand distal to the knob. FIGURES 5 and 6 represent two modified abnormal chromosomes 10 which originated from the ring chromosome. FIGURE 5 is homozygous knob$^{\circ}$ ($K^{\circ}$). This chromosome possesses the three characteristic chromomeres of the abnormal 10 but is deficient for the entire knob and distal euchromatic strand. Note that the next to last chromomere of the long arm is the one shown by an arrow in FIGURE 2. FIGURE 6 is homozygous knob$^{s}$ with the three prominent chromomeres of abnormal 10. This chromosome lacks a portion of the knob and the strand distal to the knob.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chromatid of the dyad moves to the pole more rapidly than the knobless one since the knobbed chromatid possesses neocentromeres. As a result the knobbed chromatid lies nearer to the pole than does the knobless one. This orientation is maintained until the second metaphase stage of megasporogenesis where the two spindles are arranged in tandem. At anaphase II the knobbed monads pass to the terminal poles while the knobless monads migrate to the inner poles. In maize only the basal cell survives to form the functional megaspore. Consequently, the knobbed chromosome is expected to be recovered more often than its knobless homologue, since it moves precociously to the terminal poles. On this hypothesis, therefore, preferential segregation takes place only when crossing over occurs and if neocentromeres are present.

Before discussing the evidence that supports the foregoing hypothesis of preferential segregation, it is necessary to consider one other aspect of abnormal 10. The work of LONGLEY (1945) demonstrated that knobbed chromosomes other than abnormal 10 segregated preferentially at megasporogenesis when the plants were heterozygous for abnormal 10. It was shown that non random segregation occurred only if the non-homologous chromosome was heteromorphic, as was the case for abnormal 10. He analzed preferential segregation of chromosomes 6 and 9. RHOADES and DEMPSEY (1957) reported that non random segregation occurred in plants heterozygous for knobbed chromosomes 3 and possibly 8. In other experiments RHOADES (1955b) and RHOADES and DEMPSEY (1957) showed that preferential segregation of knobbed chromosome 9 marked with the *wd* character not only took place in plants heterozygous for abnormal 10 but also in homozygous plants.

In 1942 RHOADES reported that the extent of preferential segregation depended on the map position of the locus. Subsequent experiments by RHOADES and DEMPSEY (1957) have confirmed and extended the data. They found that loci more distant from the centromere and closer to the knob underwent a higher degree of preferential segregation than did loci less favorably situated.

More recently RHOADES (1958) presented supporting evidence for the relation of crossing over to preferential segregation. This experiment was performed by using a structural abnormality in the short arm of chromosome 9 which was known to cause a great reduction in the normal amount of crossing over. On his hypothesis that preferential segregation occurs only when crossing over takes place, it follows that any event reducing the normal frequency of crossing over would also reduce the degree of preferential segregation. This proved to be true in tests of plants heterozygous for abnormal 10 with the structurally modified chromosome 9 marked with the *yg* locus, which is situated near the terminal knob on 9S, and the knobless homologue marked with the *Yg* allele. The control data showed 65.2 percent preferential segregation of the knobbed chromosome, while the data of modified-9/knob-9 compound exhibited only 54.7 percent preferential segregation of the knobbed chromosome. These data show beyond doubt that preferential segregation is greatly affected by the amount of crossing over.

Recently KIKUDOME (1958), who studied the effects of heterozygous abnormal 10 on preferential segregation of chromosome 9, found that the degree of preferential segregation was greatly influenced by the size of the knob on chromosome 9.

*Source of modified chromosomes 10*

It was reported in a previous study (EMMERLING 1955) that a large ring chromosome involving nearly the whole of abnormal 10 was produced in the progeny of plants exposed to an X-ray dosage of 400r units (Figure 4). As is shown schematically in Figure 16A, the initial break points must have occurred close to the distal end of the short arm and within the knob, so that the ring chromosome included approximately one half of the knob and the three prominent chromomeres of the long arm of abnormal 10. As a result, the ring chromosome is deficient for the distal part of the knob, the euchromatic segment distal to the knob, and the tip of the short arm. With the exception of the latter deficiency, the segment lacking in the 10L region of the ring does not represent a true deficiency for this is extra chromatin not present in a normal knobless 10. A number of ring/rod heterozygotes were examined at pachynema in order to determine the extent of the deficiency in the region of the "short arm" of the ring. A deficiency was not found. In each figure the homologous rod chromosome was paired in its entire length with the ring chromosome. However, it must be assumed that the ring chromosome possesses a small deficiency at the distal end of 10S, since two broken ends are necessary to produce a ring-shaped chromosome.

In the backcrossed progeny of plants heterozygous for the ring chromosome carrying $R^r$ (colored seed and plant) and the normal rod homologue carrying $r^g$ (colorless seed and plant), a number of exceptional plants occurred which exhibited stable plant color. Ordinarily, plants of ring-$R^r$/rod-$r^g$ constitution show variegated plant color, due to the unstable behavior of a ring chromosome. Cytological analysis of these exceptional plants revealed that the ring chromosomes had been converted to stable rods.

SCHWARTZ (1953), who encountered a similar situation in a ring-6/rod heterozygote, suggested that the ring to rod conversion arose as a consequence of crossing over in the heterozygote. If the dicentric chromatids produced by crossing over broke at anaphase, the broken ends would heal to form stable rods. A number of Drosophila workers (MORGAN 1933; STURTEVANT and BEADLE 1936; NOVITSKI 1951) have obtained similar results following crossing over in ring/rod heterozygotes.

Such a scheme could account for the origin of the stable rod chromosomes reported herein. Figure 16B shows only one of the four possible types of crossovers that would result in the production of a dicentrid chromatid at the anaphase I stage. Chromosomal bridges may also arise from both types of 3-strand and from 4-strand double exchanges. As shown in Figure 16B, a single crossover either in the long or short arms gives rise to a single chromatid bridge at anaphase I which is expected to break at telophase I (MCCLINTOCK 1938). The position of the

break may occur at any point between the two centromeres of the dicentric chromosome, producing both deficient and duplicated derivatives. McCLINTOCK (1941) has shown that the broken ends of the two newly formed chromosomes heal in the embryo to form stable rods, while in the endosperm they continue the bridge-breakage-fusion cycle.

Both duplicated and deficient rod chromosomes arose from the ring-10 heterozygote, of which only two will be considered in this paper. One of the modified chromosomes originated from breakage of the anaphase bridge at position 1 (Figure 16B). This chromosome which will be referred to as knob° (or $K°$) includes all that is present in the long arm of an abnormal 10 except for the knob and the distal euchromatic segment (Figure 16C, Figure 5). That is, the $K°$ chromosome possesses the three adjacent chromomeres characteristic of an abnormal 10 and the strand distal to this which terminates at the base of the knob. Since the $K°$ chromosome lacks the knob of abnormal 10, it could be confused with a normal knobless chromosome 10 in homozygous $K°$ plants if it were not for the presence of the three prominent chromomeres (see Figures 3 and 5 for comparison). The $K°$ chromosome is apparently free from a gross aberration either in the distal region of the short arm or in the region proximal to the last deeply stained chromomere of the long arm. Several pachytene figures of $K°$ paired with its normal knobless homologue or with an unchanged abnormal 10 failed to reveal either a deficiency or duplication.

A second type of modified abnormal 10 originated from breakage of the anaphase bridge at position 2 (Figure 16B). This chromosome which is designated knob$^s$ (or $K^s$) possesses the region of the dissimilar chromomeres and approximately one half of the heterochromatic substance (Figure 16D and Figure 6). It differs from an unchanged abnormal 10 by the absence of part of the knob and the chromatin distal to the knob, and from the $K°$ chromosome by the presence of about one half of the knob. The paired regions of normal 10 and $K^s$ chromosomes showed no indication of deficiency or duplication. This is important to note since the genetic data from tests of $K^s$ with various types of homologues are not in agreement with the cytological findings. The evidence for this will be discussed later.

### *Knob symbols*

A summary of the knob symbols that will be used for chromosome 10 in the first section of this paper is as follows:

$k$ 10— a normal knobless chromosome (Figure 3).

$K$ 10— an unchanged abnormal 10 (Figures 1 and 2).

$K°$10— a modified abnormal chromosome which is deficient for the entire knob and distal euchromatic strand but possesses the characteristic chromomeres of an abnormal 10 (Figure 5).

$K^s$10— a modified abnormal chromosome which lacks part of the knob and the distal euchromatic strand (Figure 6).

In the second section the following symbols with the chromosome number will

be used to describe the type of knob at the distal end of the short arm of chromosome 9.

*k* 9— a normal knobless chromosome.

*k* (*wd*)9—a knobless 9 associated with a minute deficiency at the distal end of the short arm.

*K* 9— knobbed chromosome used by RHOADES (1955b) and LONGLEY (1945).

$K^L$ 9— a large knob employed in the present experiment (Figure 15).

### *Viability test of* K° *and* K$^s$

The transmission of the modified chromosomes through the pollen was tested by crosses of the type *r k*/*r k* × *R* modified-*K*/*r k*, in which the seeds heterozygous for the modified knobs were colored (*Rr*) while their knobless sibs were colorless (*rr*). The *R* locus is located approximately one to two crossover units proximal to the knob (RHOADES 1942). In the absence of reduced male transmission, the ears should show equal frequencies of colored and colorless seeds.

Ordinarily a test of this type provides an extremely sensitive screen to detect the slightest chromosomal abnormality. However, in the case of plants heterozygous for the modified chromosomes, the data are complicated by the fact that an unchanged abnormal 10 exhibits reduced transmission through the male gametes. In tests of type *r k*/*r k* × *R K*/*r k*, RHOADES (1942) recovered only 42 percent of abnormal 10 where 50 percent was expected. RHOADES (1942) correlates the lowered transmission of the abnormal 10 through the pollen with the extra segment of the abnormal 10, postulating hyperploidy.

In the present experiment the compound *R K*/*r k*, including an unchanged abnormal 10, yielded 1125 *R r* (*K*) and 1419 *r r* (*k*), or 44.2 percent *K*. Two different plants of the same stock were used as male parents. The data from the transmission tests of $K^s$ come from two different male stocks. One culture, in which four different pollen parents of *R* $K^s$/*r k* constitution were tested, produced 1043 *R r* ($K^s$) and 1570 *r r* (*k*), or 39.9 percent $K^s$. The second culture, in which five different male parents were tested, gave 1685 *R r* ($K^s$) and 3105 *r r* (*k*), or 35.2 percent $K^s$. The average frequency of $K^s$ transmission is 36.9 percent. In male transmission tests of K°, two *R* K°/*r k* pollen parents of one stock gave 1955 *R r* and 2018 *r r*, or 49.2 percent K°. In another stock, four *R* K°/*r k* plants yielded 1056 *R r* and 1084 *r r*, or 49.4 percent K°.

Although these results show a decrease in the rate of male transmission of $K^s$ as compared with an unchanged abnormal 10, they do not justify the conclusion that the $K^s$ chromosomes carries a factor reducing transmission until more comparable tests are made. The reason for this is that the above data come from two unrelated stocks. There is considerable evidence against the possibility of a segmental deficiency in the $K^s$ chromosome. If this chromosome possesses a gross cytological aberration, it might be expected that the pollen would exhibit a considerable amount of sterility. However, plants of $K^s$ *k* constitution showed only 8 to 10 percent abnormal pollen which is about what RHOADES (1952) found in

plants homozygous for abnormal 10. Furthermore, there is no evidence of ovule sterility in ears heterozygous for $K^8$. In addition, the fact that the $K^8$ chromosome is viable in the homozygote also argues against a gross deficiency. Consequently while it is impossible at this time to disprove the existence of an aberration associated with $K^8$, there is ample evidence to show that $K^8$ has all the chromatin possessed by a normal 10. However, in view of the genetic data to be presented in later sections, it is important to bear in mind the lowered transmission rate of the $K^8$ chromosome through the pollen.

With the $K^\circ$ chromosome, the tests showed approximately normal transmission through the male gametes. These data are of interest since it has been demonstrated by RHOADES (1942) that an unchanged abnormal 10 with the extra segment exhibited lowered male transmission. Presumably the loss of only the knob and distal chromatin restored viability through the pollen.

Additional evidence suggests that the $K^\circ$ chromosome is of normal constitution. The pollen examinations showed that abortion was not higher than in plants homozygous for normal 10; and the cytological observations failed to reveal any abnormality. Also, ears from both heterozygous and homozygous $K^\circ$ plants did not exhibit an appreciable amount of ovule sterility.

*Effect of* $K^8$ *and* $K^\circ$ *chromosomes on crossing over*

In view of the hypothesis that preferential segregation takes place only when crossing over occurs (RHOADES 1952), it is essential to test the effects of the two modified chromosomes on crossing over. It is expected on the basis of RHOADES' experiment (1958) involving a structurally modified chromosome 9 that the degree of preferential segregation would be reduced if either of the modified chromosomes ($K^\circ$ or $K^8$) is associated with an abnormality that reduces the normal amount of crossing over in chromosome 10.

The effects of the $K^8$ and $K^\circ$ chromosomes on crossing over can be measured in the *R-K* (1–2 units) and *G-R* (14 units) regions of chromosome 10 and in regions to the left of *g*. At the present time data are available only from the *G-R* segment (Table 1). With the $K^8$ chromosome, the amount of recombination was 10.3 percent which was lower than the expected frequency of crossing over in this region. Similarly with the $K^\circ$ chromosome, there was a reduction from 14 percent to 11.5 percent. Two possibilities may be mentioned as tentative explanations of the lowered frequency of recombination. The first is that the $K^8$ and $K^\circ$ chromosomes are associated with an abnormality, causing a reduction in the normal amount of crossing over. As mentioned before, the cytological observations showed no evidence of a gross aberration. Alternatively, these differences could be explained as being due to variation since crossing over in the *G-R* region was found to vary markedly in normal stocks. With normal chromosome 10 (*k k*), the frequency ranged from 11 percent to 24 percent (EMERSON, BEADLE and FRASER 1935) while in stocks with heterozygous abnormal 10 (*K k*), 14 percent to 19.2 percent was found (RHOADES 1942 and 1952). RHOADES (1942) stated that the presence of the additional segment in abnormal 10 caused no decrease in crossing over in the *g-R* region.

TABLE 1

*Effect of* K$^{s}$ *and* K$^{o}$ *on crossing over in the* G–R *region in female gametes*

| Cross | | Noncrossovers | | Crossovers | | Crossover |
|---|---|---|---|---|---|---|
| $g\ R^r\ K^s/G\ r^g\ k \times g\ r^g\ k$/same | | $g\ R^r\ K^s$ | $G\ r^g\ k$ | $G\ R^r\ K^s$ | $g\ r^g\ k$ | Percent |
| 56:353–14 | | 35 | 35 | 2 | 2 | ... |
| –10 | | 87 | 82 | 7 | 11 | ... |
| –3 | | 65 | 63 | 8 | 9 | ... |
| –9 | | 107 | 114 | 15 | 9 | ... |
| –1 | | 100 | 70 | 12 | 13 | ... |
| –13 | | 117 | 135 | 13 | 15 | ... |
| –12 | | 62 | 67 | 7 | 6 | ... |
| –6 | | 66 | 48 | 7 | 8 | ... |
| Total | 1397 | 639 | 614 | 71 | 73 | 10.3 |
| $G\ R^r\ K^o/g\ r^g\ k \times g\ r^g\ k$/same | | $G\ R^r\ K^o$ | $g\ r^g\ k$ | $g\ R^r\ K^o$ | $G\ r^g\ k$ | |
| 79:870–8 | | 107 | 103 | 12 | 12 | ... |
| –6 | | 67 | 75 | 15 | 13 | ... |
| –10 | | 65 | 55 | 7 | 6 | ... |
| –1 | | 98 | 94 | 9 | 15 | ... |
| –5 | | 81 | 76 | 6 | 12 | ... |
| –2 | | 112 | 94 | 17 | 12 | ... |
| –4 | | 112 | 100 | 14 | 11 | ... |
| Total | 1400 | 642 | 597 | 80 | 81 | 11.5 |

Consequently, it is not possible to conclude with certainty, without further crossover data from sib comparisons of abnormal and modified chromosomes 10, that the $K^s$ and $K^\circ$ chromosomes cause a reduction in crossing over.

*A. Effect of modified chromosomes on preferential segregation for the* R *allele*

1. *Tests of* K$^{s}$ *and* K$^{o}$ *chromosomes with a knobless 10:* The effect of the modified chromosomes on preferential segregation was determined in backcrossed ears produced by pollinating female plants of *R* modified-*K*/*r k* constitution by *r k*/*r k*. The relative frequency of colored and colorless seeds was used to measure the degree of preferential segregation of the altered chromosomes. The results are presented in Table 2.

TABLE 2

*The behavior of* K$^{s}$ *and* K$^{o}$ *when tested with a normal knobless chromosome 10 in crosses of* ♀♀ R K (*modified*)/r k × ♂♂ r k/r k

| Knob type | Parental culture | Total population | Numbers of Colored *R K* | Colorless *r k* | Percent *K* | $\chi^2$ (50:50) | p |
|---|---|---|---|---|---|---|---|
| *R K* (unchanged)/*r k* | 77:1661C | 3,397 | 2,384 | 1,013 | 70.2 | ... | ..... |
| *R K*$^{s}$/*r k* | 77:1661B–62C | 14,967 | 7,433 | 7,534 | 49.7 | ... | ..... |
| | 79:1051B | 25,147 | 12,420 | 12,727 | 49.4 | ... | ..... |
| Total | | 40,114 | 19,853 | 20,261 | 49.5 | 0.96 | 0.50–0.20 |
| *R K*$^{o}$/*r k* | 77:1664A–D | 10,278 | 5,432 | 4,846 | 52.7 | 33.42 | <0.01 |

$K^s$ k *Test*: Plants of *R* $K^s/r$ *k* type gave approximately 49.5 percent of $K^s$ in separate experiments, conducted during two growing seasons. This percentage does not differ significantly from 50 percent (p = 0.5–0.2). Control data from a closely related stock had exhibited 70 2 percent of abnormal 10.

The apparent absence of preferential segregation in $K^s$ *k* heterozygotes cannot be satisfied by a simple hypothesis, since the evidence presented here are inconsistent with data to be given in later sections. However, if these data are considered apart from other tests involving $K^s$, two alternative mechanisms could account for the finding of normal backcross ratios in plants heterozygous for $K^s$.

One interpretation is that the effect is due to an unknown factor which is causing a reduction of $K^s$ transmission through the female gametes. It has been previously demonstrated that there was a probable reduction of $K^s$ transmission through the pollen. If such be the case in the female, then the ears should exhibit approximately 20 percent of ovule sterility. A number of ears were examined for ovule abortion but no appreciable amount was found. However, a small amount of sterility might not be detected unless numerous ovule counts were made.

A second explanation of the normal 1:1 backcross ratios from $K^s$ *k* plants is that crossing over was suppressed in chromosome 10. As mentioned before RHOADES (1958) reported that preferential segregation was lowered when crossing over was also reduced. If for some reason the presence of the $K^s$ chromosome inhibited crossing over throughout the entire arm of 10L the ratio of $K^s$:*k* should be 1:1 instead of 0.7:0.3. From the evidence available at present there is a possibility that crossing over is decreased in the *G-R* region from 14 to 10.3 percent but there is not the complete suppression which would be necessary in order to account for the observed 1 $K^s$: 1 *k* ratios. Crossover data from the *R-K* segment have not yet been obtained. Consequently, the interpretation of suppressed crossing over lacks sufficient support from the data on hand but cannot be excluded.

The interpretation that a factor for preferential segregation is missing from the $K^s$ chromosome is immediately excluded on the basis of the data from plants heterozygous for $K^\circ$. It follows that if the preferential segregation factor were present in either the distal one half of the knob or in the euchromatin distal to the knob, then the $K^\circ$ chromosome without these segments should not segregate preferentially. The results show, however, that $K^\circ$ segregates preferentially.

Since the genetic data exhibit no evidence of preferential segregation of $K^s$ in the heterozygote, it was of interest to examine these plants cytologically in order to determine whether neocentromeres were formed. The expectation here is that the neocentric activity would be absent since preferential segregation did not occur. Before describing the cytological observations, a word of caution must be added. The absence of preferential segregation in plants of $K^s$ *k* constitution does not necessarily mean that the $K^s$ chromosome behaves normally in other chromosomal combinations. It will be shown that $K^s$ behaves similar to *K*10 in plants of $K^s$ *K* constitution. Consequently, the cytological evidence from $K^s$ *k* plants should be interpreted with this in mind.

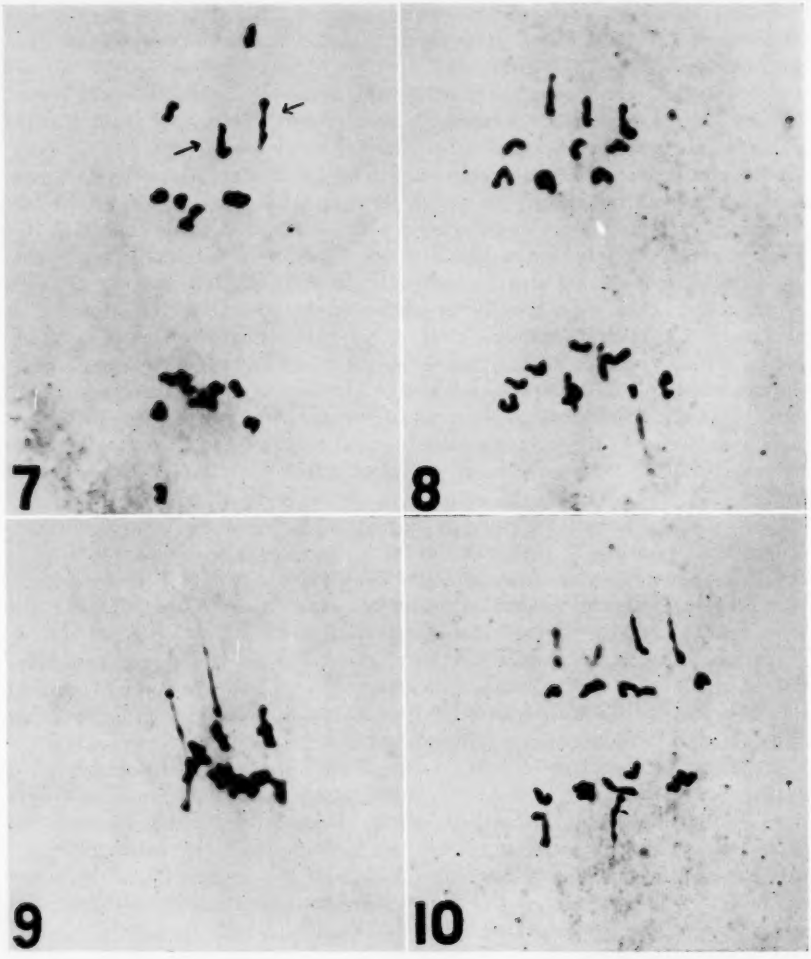
Examination of microsporocytes established that neocentromeres were formed in plants of $R\ K^s/R\ K^s$ constitution. Typical anaphase II configurations are illustrated in Figures 7 and 8. In Figure 7 it will be noted that the arms of two of the monads in the upper group are moving poleward in advance of the true centric regions. Figure 8 shows neocentric regions in three monads migrating to the upper pole and in three monads moving to the lower pole.

From these observations there appears to be a reduction in the degree of neocentric activity of $K^s$ when compared with an unchanged abnormal 10 (see RHOADES 1952 for figures of neocentric activity in plants with abnormal 10). This difference may be due to variation introduced by the stocks, or to an effect of the $K^s$ chromosome. There is ample evidence to prove that the extent of neocentric activity varies markedly in stocks heterozygous and homozygous for abnormal 10. When a random sample of six plants carrying heterozygous abnormal 10 was examined, only two of the six plants had neocentromeres, while the other four plants which showed genetic evidence of preferential segregation were without neocentromeres. In view of this variation it will be necessary to compare the degree of neocentric activity in sib plants of $K^s\ k$, $K\ k$, and $k\ k$ types before any conclusion can be drawn in regard to the apparent reduction observed in $K^s$.

Consequently, from the limited cytological information available on the neocentric activity of the $K^s$ chromosome, we can be reasonably certain of only one conclusion: some degree of neocentric activity occurs in the absence of preferential segregation. This evidence does not disprove RHOADES' hypothesis on the role of neocentromeres in preferential segregation, but it is inconsistent with it. It is conceivable that a certain amount of neocentric activity is required before the chromosomes will segregate preferentially. If such be the case, the cytological results reported here would be without much significance unless the apparent reduction of neocentric activity proves to be invalid.

K° k *Test:* When plants of $R\ K^\circ/r\ k$ type were used as the female parent in backcrosses, the percentage of $K^\circ$ transmission was 52.7 (Table 2). This differs significantly from the normal 50 percent expected on basis of random segregation of the two types of chromosome 10, and also from the 70 percent predicted on the basis of preferential segregation of the knobbed chromosome. Thus the result shows that the $K^\circ$ chromosome is able to segregate preferentially, although at a much reduced rate.

It is obvious that the data reported here are inconsistent with the data of $K^s$, as shown in the previous section. In the case of $K^s\ k$ plants, in which the modified chromosome possesses not only the three characteristic chromomeres but also part of the knob, the two types of chromosomes segregated wholly at random. In the $K^\circ\ k$ heterozygotes, in which the entire knob 10 is missing, the $K^\circ$ chromosome segregated preferentially in 52.7 percent of the functional megaspores. Why should $K^\circ$ segregate preferentially if $K^s$ does not, for the latter has more of the original abnormal 10 than the $K^\circ$? This immediately raises the question as to whether or not the data from the $K^s\ k$ heterozygote represent an actual lack of




FIGURES 7–10.—Show neocentric activity in plants homozygous for the modified chromosome 10. FIGURE 7 shows an anaphase II cell in a plant homozygous for $K^s$. The two monads which exhibit neocentric regions are indicated by arrows. FIGURE 8 is another anaphase II cell in a plant homozygous for $K^s$. In this cell, six monads possess neocentromeres of which three are migrating to the upper pole and three to the lower pole. FIGURE 9 is metaphase II in a plant homozygous for knob°. The first dyad from the left has formed neocentromeres in each arm; also one other monad shows a neocentric region. FIGURE 10 is early anaphase II in a plant homozygous for knob°. Neocentric regions are exhibited by four monads directed to the upper pole and by two to the lower pole.

preferential segregation on the part of the $K^s$ chromosome, or if some other mechanism is involved which is masking the true behavior of $K^s$. Unfortunately, this cannot be answered from the data available at the present time.

The results from plants of $K^\circ$ $k$ constitution are of interest for two other reasons: (1) the formation of neocentromeres occurred in the absence of the heterochromatic knob; and (2) a slight degree of preferential segregation also occurred in the absence of the knob and distal euchromatic segment. These two points will be discussed in the following paragraphs.

Cytological examination showed that neo-centromeres were formed in plants of $R\ K^\circ/R\ K^\circ$ type. Figure 9 illustrates a typical metaphase II stage in which two dyads possess neocentric regions. Figure 10 shows an early anaphase II stage in which four monads exhibit neocentric activity. The fact that neocentromeres are formed in the absence of the large heterochromatic segment is of interest, since it was suspected that the knob plays an important role in their production (RHOADES 1952). The observations of $K^\circ$ plants do not necessarily indicate that the knob is without function in the formation of neocentromeres. It will be seen in the photomicrographs of $K^\circ$ that the degree of neocentric activity, as indicated by the number of dyads with neocentric regions and the extent of attenuation of the chromosomal arms, is not as great as it appears in plants with abnormal 10 (see RHOADES 1952) but is similar to what was found with $K^s$. It is conceivable that the absence of the knob in the $K^\circ$ chromosome, while not completely inhibiting the expression of neocentric activity, has the effect of diminishing it. That is, the presence of only the three prominent chromomeres of the $K^\circ$ chromosome may not be sufficient for the maximum expression of activity.

Still another interpretation, that of variation, can be considered as applicable to the discussion on the reduced neocentric activity in the $K^\circ$ chromosome. This possibility has been mentioned previously in connection with the apparent reduction of neocentric activity in the $K^s$ chromosome. To test this possibility it would be desirable to examine a number of $K^\circ$ stocks of diverse background in order to determine whether any one stock exhibits more neocentric activity than another. It would also be essential to make sib comparisons of the degree of neocentric activity in $K^\circ$ and in abnormal 10 plants.

Until these tests have been completed, both alternative interpretations—actual reduction and variation—are equally compatible with the present cytological observations. It can be concluded, however, that some degree of neocentric activity occurs in the absence of the knob. Whether the segment bearing the three deeply staining chromomeres carries an agent responsible for the production of neocentromeres is unknown. Preliminary examinations of an abnormal chromosome 10 which lacks this segment but has the normal knob and the strand distal to the knob, showed no neocentric activity in 390 metaphase II and 41 anaphase II cells. However, cytological observations of one sib plant carrying a heterozygous abnormal 10 without the deficiency also exhibited no neocentric activity in 344 metaphase II cells. In view of the absence of neocentromeres in a sib plant it

becomes less certain that the lack of neocentromeres in plants carrying a deficiency for the three chromomeres is of any significance.

Another observation of interest in the $K^{\circ}$ $k$ study was the fact that the $K^{\circ}$ chromosome segregated preferentially in 52.7 percent of the functional megaspores. These data could be interpreted to mean that the heterochromatic segment and possibly the euchromatic strand distal to the knob, which are missing in the $K^{\circ}$ chromosome, have a pronounced effect on the degree of preferential segregation. Without this additional chromatin the maximum amount of preferential segregation may not be manifested. Another interpretation is that the $K^{\circ}$ chromosome is associated with an aberration which is reducing its viability. This is rather unlikely for the reason that male transmission tests and pollen examination provide no such evidence. Also, no appreciable amount of ovule sterility was found on $K^{\circ}$ $k$ ears. There is also the possibility that crossing over was reduced in the $G$-$R$ region, but this could be explained on the basis of variation. Thus it seems probable that the knob and the distal euchromatic strand play an important role in the manifestation of preferential segregation.

2. *Tests of* $K^{8}$ *and* $K^{\circ}$ *chromosomes with abnormal 10:* In experiments designed to test the behavior of the modified chromosomes 10 with an unchanged abnormal 10, plants of $R^{r}$ modified-$K$/$R^{r}$ modified-$K$ type were crossed with a stock homozygous for abnormal 10 and $R^{st}$, a stippled $R$ allele. These $F_1$ plants of $R^{r}$ modified-$K$/$R^{st}$ abnormal-$K$ constitution were used as the female parent and were backcrossed to a pollen parent homozygous for $r^{g}$ $k$. In crosses of this type, the fully colored seeds identified the modified knobbed chromosome while the stippled seeds identified the abnormal 10. Where the classification of seed color was questionable, the type of plant color (which is closely linked with seed color) was used to identify the two chromosomes; the $R$ allele of the modified knob was linked with colored plant, while the $R^{st}$ allele of the abnormal knob was without plant color.

*Control Test:* One type of control cross, in which the abnormal 10 carried $R^{st}$ and the normal knobless 10 carried $r$, gave 70.2 percent preferential segregation of the knobbed chromosome (Table 3). Data from a second type of control cross,

TABLE 3

*The behavior of* $K^{8}$ *and* $K^{\circ}$ *when tested with an unchanged abnormal chromosome 10 in crosses of* $R^{r}$ K (*modified*)/$R^{st}$ K (*unchanged*) × r k/r k

| Knob type | Parental culture | Total population | Numbers of Unchanged $K$ ($R^{st}$ $K$) | Numbers of Knobless ($r$ $k$) | Percent unchanged $K$ | $\chi^2$ (50:50) | p | $\chi^2$ (70:30) | p |
|---|---|---|---|---|---|---|---|---|---|
| $R^{st}$ $K$ (unchanged)/$r$ $k$ | 57:144 | 8,297 | 5,825 | 2,472 | 70.2 | .... | ... | .... | ... |
| | | | Unchanged $K$ ($R^{st}$ $K$) | Modified $K$ ($R^{r}$ $K^{*}$) | | | | | |
| $R^{st}$ $K$ (unchanged)/$R^{r}$ $K^{8}$ | 57:142 | 8,845 | 4,798 | 4,047 | 54.3 | 63.76<0.01 | | .... | ... |
| $R^{st}$ $K$ (unchanged)/$R^{r}$ $K^{\circ}$ | 57:143 | 10,284 | 6,659 | 3,625 | 64.8 | 895.10<0.01 | | 134.92<0.01 | |

* $K$ represents modified knob chromosomes 10.

in which the same knobbed $R^{st}$ chromosome was tested with another abnormal 10, are not available at this time. RHOADES (1952), who tested a compound of $R\,K/r\,K$ constitution found normal 1:1 ratios for the $R$ locus. As mentioned before, he concluded that preferential segregation of chromosome 10 does not occur in the homozygote ($K\,K$).

K$^8$ K *Test:* A photomicrograph of a $K^8$ chromosome paired with an abnormal 10 is shown in Figure 11. This configuration reveals the three homozygous chro-

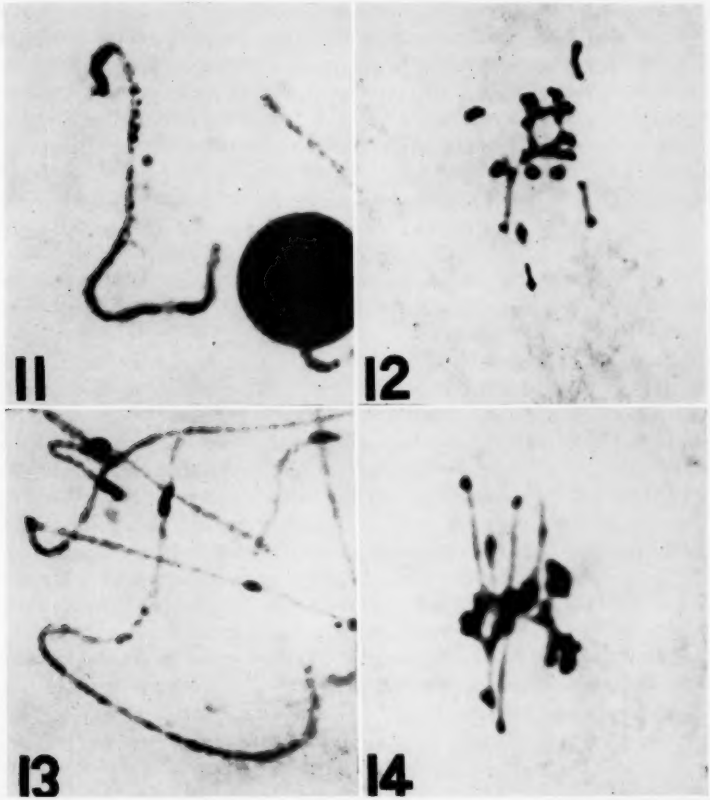



FIGURE 11.—Shows abnormal 10 paired with knob$^8$. Note that synapsis is normal throughout the entire length of the chromosomes.

FIGURE 12.—Early anaphase II in a plant with an abnormal 10 and knob$^8$ chromosome. Neocentric regions are exhibited by two to three monads going to the lower pole and one to the upper pole.

FIGURE 13.—Represents abnormal 10 paired with knob°. This figure shows that no gross abnormality is present in the knob° chromosome since pairing is normal.

FIGURE 14.—A metaphase II cell from a plant with an abnormal 10 and knob° chromosome.

momeres of the long arm, as well as the absence of a structural modification in the $K^8$ chromosome for pairing is normal in its entire length. The strand distal to the knob appears double rather than single in this particular figure. The reason for this is that in maize the two chromatids comprising each chromosome can often be seen in unpaired regions.

The results from tests of the $K^8$ $K$ compound are given in Table 3. The percentage of abnormal $K$ transmission was 54.3 which was wholly unexpected on the basis of previous evidence. It will be recalled that in the $K^8$ $k$ test, the $K^8$ chromosome behaved similar to a normal knobless 10 for it did not segregate preferentially. Accordingly, if the $K^8$ chromosome is without effect on preferential segregation in the heterozygote, it might be expected that it would show no effect when tested with an abnormal 10. If such be the case, then the expected frequency of abnormal 10 would be 70 percent, since $K^8$ $K$ should act similarly to $K$ $k$. On the other hand, if for some unknown reason both knobs of the $K^8$ $K$ compound were equally effective, then the expected frequency would be 50 percent, as shown by RHOADES (1952). The data in Table 3 show that the ratio of $K10$:$K^8$ is not greatly different from a normal 1:1 ratio. This definitely does not support the previous suggestion that the $K^8$ chromosome is without the ability to segregate preferentially. The slight excess of abnormal 10 above 50 percent could be ascribed to either megaspore competition between the two types of knobbed chromosomes, or to a reduction in viability of the $K^8$ chromosome.

Cytological examinations of $K$ $K^8$ plants exhibited neocentric activity at the metaphase II and anaphase II stages. Figure 12 illustrates an early anaphase II cell in which only a few of the monads exhibit neocentromeres. In this compound it might be expected that the neocentric activity would be greater since an abnormal 10 is present. However, the configurations of this particular stock displayed reduced neocentric activity, similar to that which was found for $K^8$ $k$ and $K^\circ$ $k$.

K° K *Test*: A pachytene configuration of a $K^\circ$ chromosome paired with abnormal 10 is shown in Figure 13. This photomicrograph shows that no gross cytological aberration is present in the $K^\circ$ chromosome for pairing is normal. It will be also noticed that the three chromomeres of the long arm are homozygous, and that the chromosomes appear double up to the knob which is in agreement with Figure 5. The most distal chromomere in Figure 13 corresponds to the second most distal chromomere in Figure 5. The last chromomere in Figure 5 is apparently either the last chromomere adjacent to the knob, which is not visible in Figure 13, or a small fraction of the knob.

The genetic results in Table 3 show that 64.8 percent of the backcross progeny of $K^\circ$ $K$ plants possess the abnormal 10. As explained before in connection with the data from $K^8$ $K$ heterozygotes, the expected frequency of abnormal 10 in the presence of two equally effective knobs is 50. In cases where one knob is less effective than the other, the percentage of $K$ is predicted to lie between 50 and 70. Inspection of the data of $K^\circ$ $K$ compounds demonstrate that while $K^\circ$ is included

less often in the functional megaspores than $K$, it is not wholly without some capacity to compete with abnormal 10. If the $K^\circ$ chromosome were without any effect, then approximately 70 percent of the functional megaspores should include abnormal 10 instead of the 64.8 percent which was observed. It could be argued that the difference between 64.8 and 70 percent is not real since sib comparisons were not made. However, the data from the $K^\circ$ $k$ test are against this view. It was shown in Table 2, that when $K^\circ$ was tested with a normal chromosome, 52.7 percent of the functional megaspores included $K^\circ$. Consequently, the $K^\circ$ chromosome should also exhibit a slight degree of preferential segregation in the presence of an abnormal 10, which it did. For this reason the results from $K^\circ$ $K$ are not unexpected and are consistent with the previous data of $K^\circ$ $k$.

The extent of neocentric activity observed in $K^\circ$ $K$ plants (Figure 14) was much the same as found in $K^s$ $k$, $K^\circ$ $k$, and $K^s$ $K$ plants. Here again the degree of neocentric activity appears reduced as compared with homozygous abnormal 10 (see RHOADES 1952).

3. *Test of* K° *with* K$^s$: The results from crosses of type $R\ K^\circ/r\ K^s \times r\ k/r\ k$, in which the two modified chromosomes were tested against each other, are presented in Table 4. The data show that the $K^\circ$ chromosome was included in approximately 51.9 percent of the functional megaspores, a value which deviates significantly from 50 percent. Thus the $K^s$ chromosome is less effective than the $K^\circ$ chromosome.

### *B. Effect of modified chromosomes 10 on preferential segregation of chromosome 9*

The effect of the $K^s$ and $K^\circ$ chromosomes on preferential segregation of heteromorphic chromosome 9 bivalents was studied by using RHOADES' method (1955b). Plants which were heterozygous for modified chromosomes 10 and for knobbed chromosome 9 were backcrossed as the female parent by a stock homozygous for knobless chromosomes 9 and 10. The degree of preferential segregation for the knobbed chromosome 9 was measured by using the *wd* character which is a minute deficiency for the tip of the short arm of chromosome 9 (MCCLINTOCK 1944). The advantage of using this deficient chromosome as a marker is that it is always associated with the knobless chromosome 9, and it has no effect on viability. The knobbed chromosome 9 used in these tests carried the marker *Wd* and a large terminal knob ($K^L$) on the end of the short arm (Figure 15).

TABLE 4

*The results from crossing females heterozygous for* K° K$^s$ *to males homozygous for knobless 10* (♀♀ R K°/r K$^s$ × ♂♂ r k/r k)

| Numbers of | | | | | |
|---|---|---|---|---|---|
| Total population | Colored ($R\ K^\circ$) | Colorless ($r\ K^s$) | Percent $K^\circ$ | $\chi^2$ | P |
| 12,153 | 6,308 | 5,845 | 51.9 | 17.62 | <0.01 |

FIGURE 15.—A chromosome 9 with a large terminal knob on the short arm.

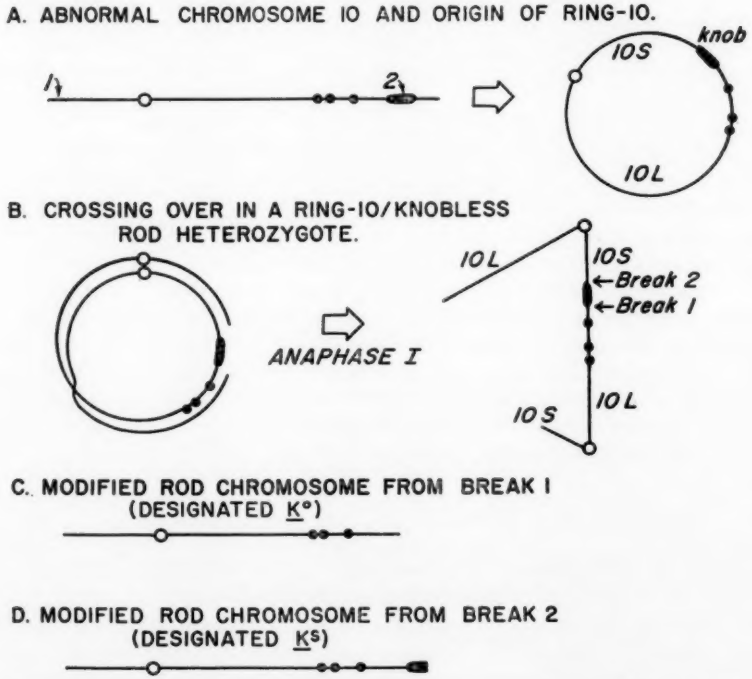



FIGURE 16.—The origin of two modified abnormal chromosomes 10 resulting from crossing over in a ring-10/rod heterozygote. The ring chromosome includes the three prominent chromomeres of an unchanged abnormal 10 and approximately one half of the original knob.

*Test of* K$^{s}$: In the test with the $K^{s}$ chromosome, the cross was of the following type: $K^{s}10/k10$, $K^{L}9$ $Wd/k9$ $wd \times k10/k10$, $k9$ $wd/k9$ $wd$. The data are summarized in Table 5. These results show that the knobbed chromosome 9 carrying *Wd* was included in 53.5 percent of the functional megaspores, a value which deviates significantly from 50 percent at the one percent level. Thus $K^{s}$ 10 has some effect on preferential segregation of $K^{L}$ 9. Had the $K^{s}$ chromosome been without any effect on chromosome 9, the percentage of *Wd* should have been 50, for a heterozygous knobbed chromosome 9 does not segregate preferentially in the absence of abnormal 10 (RHOADES 1955b). If, on the other hand, the $K^{s}$ chromosome were wholly efficacious, then approximately 68 percent of the functional megaspores would include the $K^{L}$ 9. This percentage is based on the 1958 data of KIKUDOME who used the same sized knob on chromosome 9 as was employed in the tests of the $K^{s}$ chromosome.

*Test of* K°: The data obtained from tests of $K^{\circ}$ on preferential segregation of the knobbed chromosome 9 are presented in Table 5. The cross is the same as that described in the previous section. The result shows that 53.2 percent of the functional megaspores received the $K^{L}$ 9 chromosome. This percentage deviates significantly from 50 at the one percent level.

The effect of $K^{\circ}$ on $K^{L}$ 9 was low, as might be expected, since the other tests involving $K^{\circ}$ also showed a low degree of preferential segregation. It will be recalled that in the heterozygote ($K^{\circ}$ $k$) only 52.7 percent of $K^{\circ}$ was found instead of the usual 70 percent. Similarly, in the case of the $K^{\circ}$ $K$ analysis, 35.2 percent of $K^{\circ}$ was recovered rather than the normal 50 percent. Therefore, the data presented here are consistent with the previous findings.

From these observations it is possible to conclude that the $K^{\circ}$ chromosome has some effect on the segregation of the knobbed chromosome 9. This is of interest since previous workers (LONGLEY 1945; RHOADES 1955b; RHOADES and DEMPSEY 1957; KIKUDOME 1958) have shown that preferential segregation of other knobbed chromosomes occurred only in the presence of abnormal 10 with the large heterochromatic segment. The $K^{\circ}$ chromosome which lacks this knob is still capable of influencing the behavior of chromosome 9. However, the effect is greatly reduced, a fact which could be attributed to the absence of the heterochromatic segment.

TABLE 5

*The effect of* K$^{s}$ *10 and* K$^{\circ}$ *10 on the behavior of the knobbed chromosome 9 in crosses of* ♀♀ *modified* K *10*/k *10*, K$^{L}$ *9*/k *9* × ♂♂ k *10*/k *10*, k *9*/k *9*

| Knob type | Parental culture | Total population | *Wd* $K^{L}$ 9 | *wd* *k* 9 | Percent *Wd* | $\chi^{2}$ 50:50 | p |
|---|---|---|---|---|---|---|---|
| $K^{s}$ 10/*k* 10, *Wd* $K^{L}$ 9/*wd* *k* 9* | 57:158 | 4,172 | 2,233 | 1,939 | 53.5 | 20.72 | <0.01 |
| $K^{\circ}$ 10/*k* 10, *Wd* $K^{L}$ 9/*wd* *k* 9 | 57:159 | 5,337 | 2,840 | 2,497 | 53.2 | 22.04 | <0.01 |

* Knobbed chromosome 9 was marked for *Wx* in 2338 of the 4,172 gametes tested. The $K^{L}$ 9 chromosome showed approximately 52 percent *Wx*.

TABLE 6

*The summarized results of the modified chromosomes tested with a normal knobless chromosome 10, an abnormal 10, and a knobbed chromosome 9*

| | Female transmission | | | Male transmission |
|---|---|---|---|---|
| | Knobless 10 | Abnormal 10 | Knob$^L$ 9 | Knobless 10 |
| Abnormal 10 | 70.2 | 50.0* | 68.0† | 44.2 |
| Knob$^8$ 10 | 49.5 | 54.3 | 53.5 | 36.9 |
| Knob$^o$ 10 | 52.7 | 64.8 | 53.2 | 49.3 |

$K^8/K^o$—51.9 percent, $K^o$.
*G–R* recombination in $K^o\,k = 10.3$ percent.
*G–R* recombination in $K^8\,k = 11.5$ percent.

* Data from RHOADES (1952).
† Data from KIKUDOME (1958).

## SUMMARIZED RESULTS

The summarized results of the modified chromosomes tested with a normal knobless chromosome 10, an abnormal 10, and a knobbed chromosome 9 are shown in Table 6. In the first three columns the percentage of female transmission (or preferential segregation) are recorded. Column 1 shows the results from tests of the various knobbed chromosomes 10 with a normal knobless chromosome. Column 2 gives the percentages of abnormal 10 resulting from trials with the modified chromosomes. In column 3 the effect of the modified chromosomes on a knobbed chromosome 9 are recorded in terms of the frequency of knobbed chromosome 9 segregation. The last column refers to the percentages of the various knobs resulting from male transmission tests. Immediately below the table the frequency of $K^\circ$ in $K^\circ$ $K^8$ heterozygotes is given. The percentages of recombination in the *G-R* region in plants of $K^\circ$ $k$ and $K^8$ $k$ constitution are also shown below the table.

## SUMMARY

(1) An analysis was made of the effects of two structurally modified chromosomes 10 on preferential segregation of chromosomes 9 and 10.

(2) The knob° chromosome, an altered abnormal 10 without the heterochromatic strand, has a reduced effect on the segregation of chromosomes 9 and 10. This suggests that the knob and the distal euchromatic thread play an important role in the manifestation of preferential segregation. The knob° chromosome also shows an apparent reduction in the degree of neocentric activity, a fact which could be ascribed to the absence of the heterochromatic segment.

(3) The knob$^8$ chromosome, an altered abnormal 10 lacking about one half of the heterochromatic segment and distal euchromatic strand, shows normal 1:1 ratios when tested with a knobless 10 but forms neocentromeres. In tests with an abnormal 10 and a knobbed chromosome 9, knob$^8$ has some effect. At the present time it is difficult to formulate a single hypothesis that would account for the contradictory results from the $K^8$ chromosome.

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